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ORIGINAL ARTICLES

CRYSTALLINE B_{12} INHALATION THERAPY IN PERNICIOUS ANEMIA*

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OF the previously suggested routes for B_{12} administration in the treatment of pernicious anemia, parenteral injection alone has thus far proved satisfactory. Oral[1,2] as well as sublingual administration has resulted in unsatisfactory clinical and hematologic responses, except when given in massive doses or in conjunction with intrinsic factor[4].

The success of parenteral injection is predicated upon the ready access of the injected material to capillaries and lymphatics. One of the largest capillary beds in the body which contacts the external environment is that of the pulmonary circulation. Inhalation therapy seemed to be a logical route for attempted administration of B_{12}. Reports of the effectiveness of aerosol therapy in other fields such as the antibiotics, hormones and vasoconstrictors lend support to the validity of this concept[2]. B_{12} inhalation therapy has resulted in adequate clinical and hematologic responses in 3 patients, 2 presenting severe and one mild manifestations of pernicious anemia.

Materials and Methods. Crystalline B_{12} in physiological saline solutions without a preservative was utilized in the first 2 cases. The material was administered initially by means of pressure tank and a vaponephrin nebulizer and contained 15 μgm. of the vitamin per 1.0 cc. The dosage was that usually employed in parenteral administration of this agent. In one patient after 9 days of administration utilizing the pressure tank, self-administration was effected by means of a hand atomizer.

In the third case, 1000 μgm. of crystalline B_{12} in 0.1 cc. by volume of lactose powder was utilized for administration as a dust. This was an attempt to further simplify self-administration. The B_{12} lactose powder was administered by means of a dust inhaler (aerohalor-Abbott). The patient was directed to take 10 deep inhalations per administration at the times corresponding to usual parenteral dosage schedules.

Later experience with this method has shown that larger amounts of active material are deposited in the lungs if a forced expiration immediately precedes a slow, deep inspiration. Immediately following this inspira-

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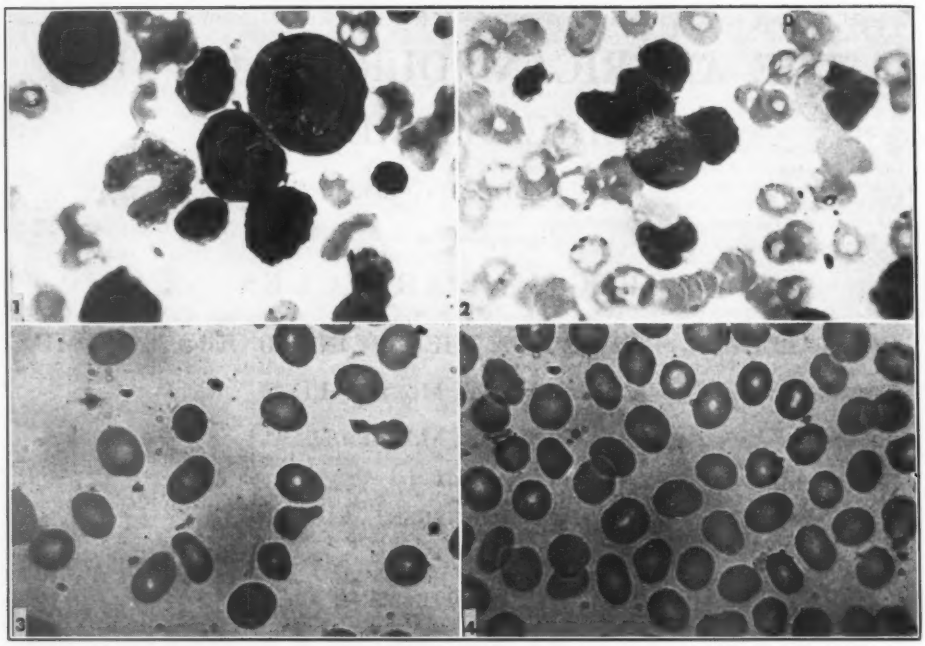

FIG. 1. CASE 1.—Representative megaloblasts and a giant neutrophilic metamyelocyte in the marrow aspirate prior to treatment. X1200.

FIG. 2. CASE 1.—Normoblasts and unaffected members of the neutrophilic series in the marrow aspirate after crystalline B_{12} aerosol treatment. X1200.

FIG. 3. CASE 1.—Megalocytes and poikilocytes in the peripheral blood prior to treatment.

FIG. 4. CASE 1.—Normocytes with only an occasional macrocyte in the peripheral blood after crystalline B_{12} aerosol treatment. X1200.

tion the breath should be held for a few moments then exhaled through the nose[6].

Case Reports. CASE 1. F. Z., HFH Case No. 674802, a white male aged 56, was first seen April 14, 1952, complaining of weakness, anorexia and exertional dyspnea of 8 weeks' duration. For several weeks he had noted numbness in the lower extremities. The past history was non-contributory.

Physical examination revealed a well-developed, well-nourished male with a lemon yellow skin color. There was moderate atrophy of the tongue and marked pallor of the mucous membranes. Neurological examination was normal except for mild hypesthesia of the lower extremities.

Pertinent laboratory data on admission included a gastrointestinal survey, including liver function tests and other biochemical blood determinations, that was normal excepting a histamine-fast achlorhydria; hemoglobin, 5.9 gm. per 100 cc.; erythrocytes 1,360,000 per c.mm.; hematocrit 17.1%; mean corpuscular volume 125.7 c.μ.; mean corpuscular hemoglobin 43.3 mμgm.; mean corpuscular hemoglobin concentration 34.5%; reticulocytes 2.2%; leukocytes 6,300 per c.mm.

The blood smear (Fig. 3) revealed a macrocytic erythrocytic picture characterized by the presence of megalocytes, poikilocytosis, anisocytosis and polychromasia. The neutrophilic leukocytes presented hypersegmentation of their nuclei.

The sternal aspirate revealed paradoxical hyperplasia and the presence of 44.2% megaloblasts and 12% giant neutrophils of the pernicious anemia type indicative of marked deficiency of anti-pernicious anemia factors (Fig. 1, Table 1).

The patient was given daily inhalations of 15 μgm. of crystalline B_{12} in 1 cc. of physiological saline. No other anti-anemic preparations were given.

The hematologic response is shown in Chart 1. On the 7th day of therapy the reticulocyte response was 21% and on the 8th day of therapy 44.2%. The patient continued on daily self-administered inhalations

TABLE 1.—DIFFERENTIAL COUNTS (1000 CELLS) OF STERNAL MARROW ASPIRATES OF PERNICIOUS ANEMIA PATIENTS BEFORE AND AFTER TREATMENT WITH CRYSTALLINE B_{12} AEROSOL AND B_{12}–LACTOSE DUST INHALATION

	Before	*After**	*Before*	*After**	*Before*	*After*
Myeloid: erythroid ratio	1.1:1	2.4:1	5:1	5.5:1	0.9:1	5.6:1
Reticulum cells (occ.)	0.4	0.5	1.0		0.6	0.4
Myeloblasts (1.)	0.2	0.4	0.4		0.1	0.4
Progranulocytes (2.)	1.4	2.8	4.2	0.6	1.3	3.7
Neutrophilic myelocytes early (4.)	4.2	5.4	4.0	2.8	4.4	5.3
Neutrophilic myelocytes late (13.)	5.6	5.4	5.4	3.8	5.2	10.0
Neutrophilic metamyelocytes (15.)	3.2	7.0	5.4	4.6	6.6	8.3
Band cells (16.)	7.2	10.6	10.4	5.0	6.7	13.9
Neutrophils segmented (15.)	12.6	26.0	29.2	35.8	5.9	24.1
Immature neutrophils, P.A. Type (0)	9.0	2.4	3.4	0.6	5.6	2.3
Neutrophils segmented, P.A. type (0)	3.0	2.0	2.6	1.2	1.3	0.1
Eosinophilic myelocytes early		0.3		0.2	0.1	0.2
Eosinophilic myelocytes late (2.)	0.4	0.8	0.8	0.2	0.4	1.6
Eosinophilic metamyelocytes	0.4	0.4	0.6		0.4	0.6
Eosinophils (1.)	1.0	0.5	2.8	0.8	0.2	1.0
Basophils, immature (occ.)		0.2	0.4			0.2
Basophils (0.5)			0.4		0.5	
Prolymphocytes (occ.)		0.1			0.3	0.6
Lymphocytes (10.)	5.2	6.5	11.2	31.4	7.2	10.6
Leukocytoid lymphocytes (0)	0.2		0.2			
Monocytes (1.)		1.7		7.2	0.1	0.9
Proplasmocyte (occ.)						0.1
Plasmocyte (1.)		0.2	1.8	0.2	0.5	0.3
Pronormoblasts (0.5)		0.7	0.8	0.2	1.3	
Basophilic normoblasts (2.)		1.8	1.4	0.6	2.7	0.8
Polychromic normoblasts (12.)		20.9	6.2	4.0	5.5	7.9
Orthochromic normoblasts (4.)	0.2	2.1	4.2	0.4	2.4	3.7
Promegaloblasts (0)	7.2	0.2			2.8	0.1
Basophilic megaloblasts (0)	17.0	0.2	0.2		21.8	0.1
Polychromic megaloblasts (0)	16.6	1.1	1.8		12.8	1.5
Orthochromic megaloblasts (0)	3.4	0.6	0.4		2.2	0.7
Phagocytic histiocytes (occ.)	0.2					0.1
Lipid histiocytes (0)						0.1
Fat cells (occ.)			Predominate			
Mitoses, white	0.2	0.1			0.5	0.1
Mitoses, red	1.2	0.9		0.4	1.4	0.3
Cellularity	Packed	Normal	Hypo-plastic	Hypo-plastic	Packed	Normal

* Three weeks after therapy

of B_{12} at home without symptoms or signs of pulmonary irritation. A continued rise in hemoglobin and erythrocyte count has been noted on return visits to the outpatient department. By the 20th day the erythrocyte count had more than doubled with an increase of over 500,000 erythrocytes per week.

Examination of the marrow aspirate (Table 1) revealed a drop in megaloblasts to only 2.1% and of pernicious anemia type neutrophils to 4.4% (Fig. 2). At this time the patient presented marked symptomatic improvement. Therapy was continued by inhalation of 15 μgm. three times a week for another month followed by inhalation of 100 micrograms in 1 cc. saline once a week by hand atomizer. By July 1st, the erythrocyte count reached 4,680,000 per c.mm., and the hemoglobin 14.5 gm.

Case 2. L. H., HFH Case No. 425149, a white female aged 71 years, was first seen on April 20, 1952. Complaints were weakness, weight loss and paresthesias of all extremities. The past history was non-contributory.

Physical examination revealed a poorly nourished, pale, elderly female. There was mild atrophy of the tongue and pallor of the mucous membranes. Neurological examination demonstrated mild peripheral neu-

ritis in both legs, considered by the neurological consultant as consistent with the early stage of subacute combined degeneration of the cord.

A gastrointestinal survey, including liver function tests, and biochemical studies of the blood were normal except for the presence of histamine-fast achlorhydria.

Pertinent hematological data on admission were as follows: Hemoglobin 12.1 gm. per 100 cc.; red cells 3,480,000 per c.mm.; hematocrit 37.8%; mean corpuscular volume 108.5 c.μ., mean corpuscular hemoglobin 31.8 mμgm.; mean corpuscular hemoglobin concentration 32.0%; reticulocytes 1.7; leukocytes 6,200 per c.mm. Examination of the blood smear revealed a macrocytic erythrocytic picture with anisocytosis and polychromasia as additional features. An occasional neutrophilic leukocyte presented nuclear hypersegmentation.

Examination of the sternal aspirate revealed hypoplasia and the presence of 2.4% megaloblasts and 6% giant neutrophils of the pernicious anemia type indicative of definite but moderate deficiency of anti-pernicious anemia factors.

The patient was given daily inhalations of 15 μgm. of crystalline B_{12} for 6 days, then, 3 times a week for 2 weeks. The hematologic response is shown in Chart 2. Three weeks after the institution of aerosol therapy the red blood cell count increased from 3,480,000 to 4,400,000 per c.mm. and the hemoglobin rose from 12.1 to 14.2 gm. There was symptomatic improvement of the peripheral neuritis and a weight gain of 10 pounds.

Case 3. S. G., HFH Case No. 686563, a white female aged 51 years, was first seen August 4, 1952, complaining of weakness and sore tongue as well as numbness and tingling of all extremities. The past history was noncontributory.

Physical examination revealed a pale, slightly obese female. A distinct yellow tint to the skin and sclerae was evident. The tongue presented a mild degree of atrophy of the lateral margins and a reddened tip. Neurological examination showed moderate hyperesthesia of the extremities.

Pertinent laboratory data on admission were as follows: a gastrointestinal survey, including liver function tests and other biochemical blood determinations, revealed cholelithiasis and a histamine-fast achlorhydria; hemoglobin, 7.5 gm. erythrocytes 1,640,000 per c.mm.; hematocrit 23.6%; mean corpuscular volume 143.8 cμ.; mean corpuscular hemoglobin 45.7 mμgm.; mean corpuscular hemoglobin concentration 31.7%; reticulocytes 1.4%; thrombocytes 100,000

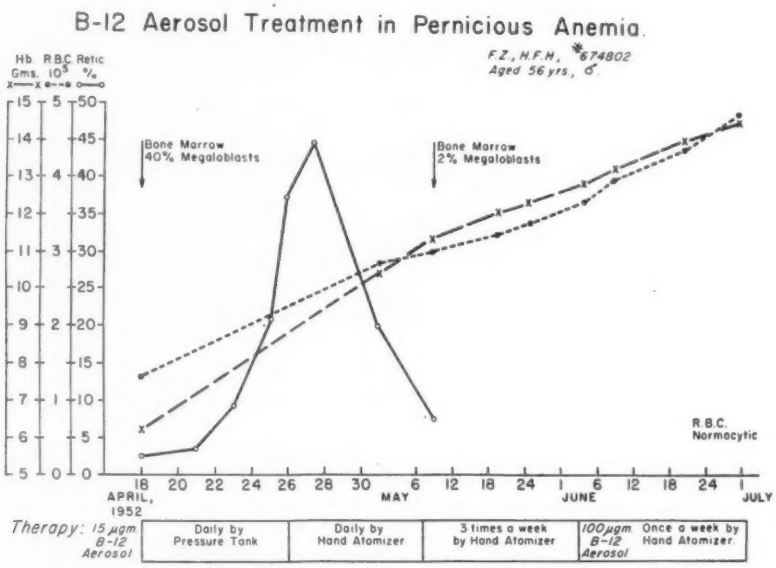

Chart 1.—Hematologic response to crystalline B_{12} aerosol treatment in pernicious anemia, Case 1.

per c.mm.; leukocytes 5,200 per c.mm. Examination of the blood smear revealed a macrocytic erythrocytic picture characterized by the presence of megalocytes, poikilocytosis, anisocytosis, polychromasia, basophilic stippling and an occasional normoblast. The neutrophliic leukocytes presented hypersegmentation of their nuclei and a slight "shift to the left."

Examination of the sternal aspirate revealed paradoxical hyperplasia and the presence of 39.6% megaloblasts and 6.9% giant neutrophils of the pernicious anemia type indicative of marked deficiency of antipernicious anemia factors (Table 1).

The patient was given daily inhalations of crystalline B_{12} in lactose powder. Ten deep inspirations per day were self-administered for 8 days. No other anti-anemic preparations were given. The hematologic response is shown in Chart 3. On the 8th day of therapy the reticulocyte response was 22.0%. Study of Chart 3 reveals a persistence of reticulocytosis above 10% for over 15 days. In the first 3 weeks of therapy the erythrocyte count increased from 1,640,000 to 2,960,000 per c.mm., or an average of over 400,000 erythrocytes per c.mm. per week. The hemoglobin rose from 7.5 to 10.4 gm. The glossitis and hyperesthesias disappeared and color and strength improved.

The patient was discharged to her home after 8 days of hospitalization with instructions to take 5 deep inspirations of B_{12} three times a week for the first week and twice a week for the second week. In the succeeding 3 week period she took only 5 inspirations of B_{12} lactose dust on one occasion. On this reduced dosage schedule the erythrocyte count dropped from 2,960,000 to 2,520,000 per c.mm. Upon reinstitution of 10 inspirations of B_{12} lactose dust three times a week, the patient again demonstrated an increase in her erythrocyte count at the rate of 500,000 cells per c.mm. per week (Chart 3). Examination of the marrow aspirate (Table 1) at the end of this period revealed a drop in megaloblasts to 2.4%. The thrombocytes had risen from the original level of 100,000 to 375,000 per c.mm.

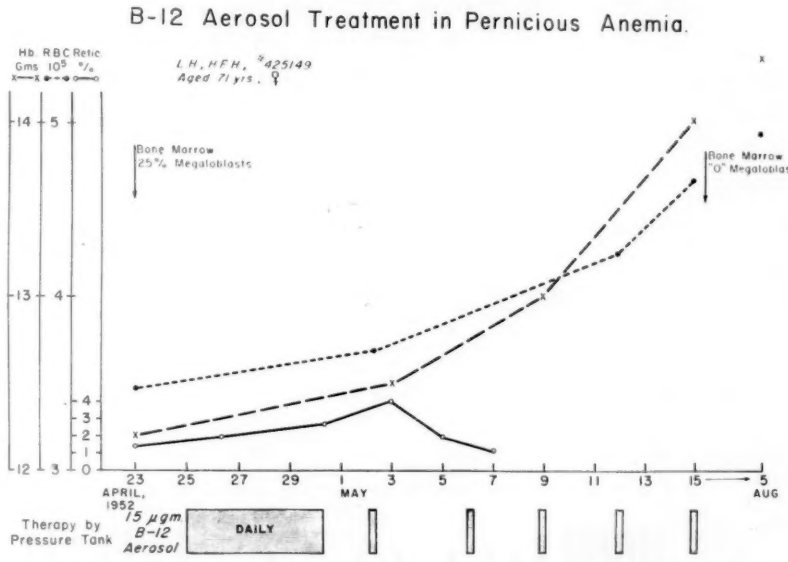

CHART 2.—Hematologic response to crystalline B_{12} aerosol treatment in pernicious anemia, Case 2.

Discussion. The suitability of B_{12} inhalation therapy requires some amplification. Few drugs now being used in inhalation therapy meet the exacting requirements of lack of toxicity and need for closely controlled dosage. Inhalation of crystalline B_{12} in physiological saline or lactose powder has, to date, produced no objective or sub-

jective evidence of toxicity or sensitivity at the pulmonary site.

There is no question but that B_{12} inhalation therapy involves additionally some degree of oral therapy including sublingual and gastrointestinal routes. However, satisfactory responses to inhalation of the vitamin were obtained at dosage levels far below those needed for effective oral therapy[4,5].

per cc.[1]. After inhalation of only 200 μgm of B_{12} in saline, B_{12} activity in the urine reached the level of 0.26 mμgm per cc., and following inhalation of approximately 500 μgm of B_{12} in lactose powder B_{12} activity in the urine rose to 0.134 mμgm per cc. Direct pulmonary instillation of 100 μgm. of crystalline vitamin B_{12} by means of the bronchoscope, resulted in a urinary excre-

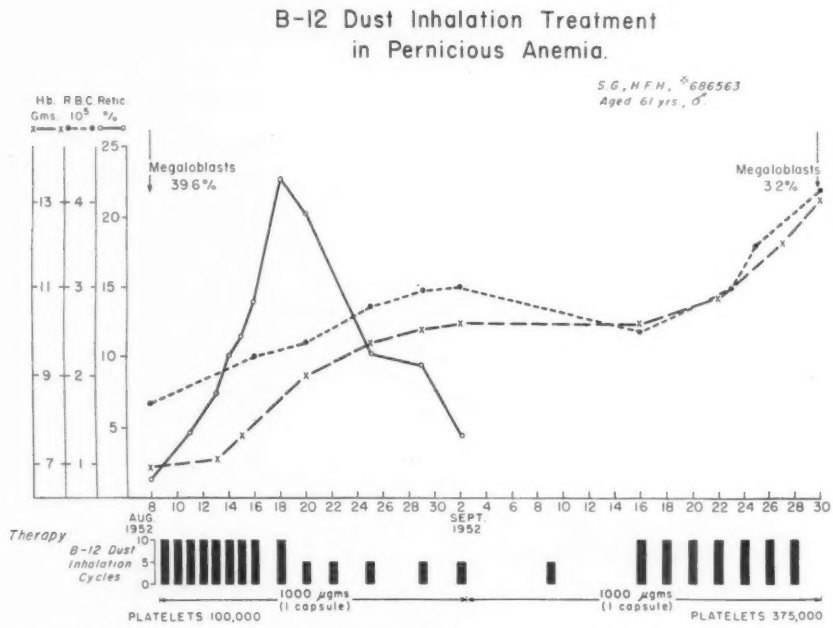

CHART 3.—Hematologic response to crystalline B_{12}-lactose dust inhalation treatment in pernicious anemia, Case 3.

To evaluate further the efficacy of pulmonary absorption of the inhaled B_{12} over and above the evidence afforded by the clinical and hematological response previously described, urinary excretion studies were performed. These latter consisted of measurement in the urine of B_{12}-like growth stimulating substance, using the test organism, *Lactobacillus leichmanii*. Upon oral administration of B_{12} at dosages as high as 10,000 μgm., B_{12} activity in urine has been shown to be less than 0.05 mμgm tion (9.76 mμgm. per cc.) pattern equivalent to the intramuscular injection of 60 to 80 μgm. of B_{12}. These results indicate a definite increase in urinary B_{12}-like growth substance in the urine of those subjects receiving B_{12} inhalation administration.

Intranasal B_{12} drops and dust, as routes of administration, are being investigated.

Although these results are uniformly encouraging, they, of course, constitute only preliminary observations as to the

efficiency of inhalation crystalline B_{12} treatment of pernicious anemia. Additional investigations will concern the problem of long-term maintenance of the pernicious anemia patient with these newer methods of specific antipernicious anemia therapy.

Summary. Inhalation therapy with crystalline B_{12} in physiological saline and lactose dust has resulted in adequate clinical and hematologic responses in 3 patients, 2 presenting severe and one mild grades of pernicious anemia. Detectable amounts of B_{12} activity in the urine were found after pulmonary administration of this substance.

ADDENDUM: Subsequent to the submission of this manuscript, 3 additional pernicious anemia patients in relapse have been adequately treated by inhalation crystalline B_{12}.

ACKNOWLEDGMENT: The authors wish to express appreciation to H. W. Schoenlein, Director of Bacteriological Laboratories, Difco Laboratories Incorporated, for supplying the crystalline B_{12}-lactose powder and the urinary B_{12} determinations.

REFERENCES

1. **Conley, C. L., Krevans, J. R., Chow, B. F., Barrows, C.,** and **Long, C. A.**: J. Lab. & Clin. Med., **38**, 84, 1951.
2. **Dautrebande, I.**: Physiol. Rev., **32**, 214, 1952.
3. **Meyer, L. M., Sawitsky, A., Chen, B. S., Krim, M.,** and **Fadem, R.**: Am. J. Med. Sci., **220**. 604, 1950.
4. **Ungley, C. C.**: Brit. Med. J., **2**, 905, 1950.
5. Idem: Nutrit. Abst. Rev., **21**, 11, 1951.
6. **Vermilye, H. N.**: J. Am. Med. Assn., **129**, 251, 1945.

TOTAL SERUM CHOLESTEROL, CHOLESTEROL-LIPID PHOSPHORUS RATIO, AND S_f 12-20 CONCENTRATION IN HYPERTENSION, DIABETES, AND CORONARY ARTERY DISEASE*

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THE increased interest recently in the role of the blood cholesterol and particularly the lipoprotein contained in the plasma in the pathogenesis of arteriosclerosis[4] has resulted in considerable controversy. In some instances the data has been interpreted to indicate that the total cholesterol determination may provide at least as useful and perhaps more useful diagnostic information as to the presence of arteriosclerosis than does the ultracentrifuge determination of the lipoprotein pattern[6]. With two exceptions[5,7], all of the reports so far appearing on the determination of the S_f 12-20 pattern have been based on determinations made by the laboratory of the group originally describing this technique[3].

This study was undertaken to determine the variations in the total serum cholesterol, S_f 12-20 lipoprotein concentration, and cholesterol-lipid phosphorus ratio in a series of patients admitted to a general hospital with findings of hypertension, diabetes mellitus, and coronary artery disease. It was felt that an independent study of these factors might lend considerable evidence to this controversial problem.

Methods. Forty cubic centimeter venous blood samples were obtained at random times during the day from patients entering the hospital with hypertension, diabetes, and coronary artery disease. Control normal subjects consisting of hospital personnel and staff were used. The criteria for inclusion in the hypertensive group were a finding of a blood pressure consistently above 145 mm. Hg systolic and/or 90 mm. Hg diastolic. Elevations of systolic blood pressure up to 150 mm. Hg were considered normal if the diastolic reading was 90 mm. Hg or below. Patients were considered to have coronary artery disease only if they demonstrated the typical history of angina of effort recurring over several months, or if there were a history and physical findings suggestive of myocardial infarction together with confirmatory serial electrocardiographic data, hemographic and sedimentation rate changes. Patients with diabetes mellitus were included only if they had incontrovertible evidence of the disease accompanied by marked fasting hyperglycemia. All but 4 of the diabetics were receiving 20 or more units of insulin per day.

Total cholesterol and cholesterol ester determinations were done by a modification of the Sperry-Schönheimer method[2]. The lipid phosphorus was determined by the method of

* Portions of this study were made possible by grants-in-aid from the Western Pennsylvania Heart Association and the Public Health Service National Institutes of Health.

Fiske and SubbaRow[1]. Lipid phosphorus values were recorded as such and not after arbitrary conversion to phospholipid. The ultracentrifugal determinations of the S_f 12-20 concentration were performed using methods developed for the United States Public Health Service cooperative study of lipoproteins in atherosclerosis.*

Of the individuals used in this study, there were 17 normal males and 13 normal females with a mean age of 33 and 36 years respectively. The male normals averaged 150 lb. in weight and the females 139 lb. The mean blood pressure levels for the males were 124/77 mm. Hg and for the females 120/78 mm. Hg.

In the 63 hypertensive males and 94 hypertensive females, the average age in both groups was 57 years. The average weight of the males was 170 pounds and the females 148 pounds. The average blood pressure of the males in this group was 180/108 and the females 186/108 mm. Hg.

In the 18 diabetic males and 35 diabetic females the average ages were 62 and 60 years respectively, the average weights were 154 and 146 lb. The blood pressures in this group averaged 152/85 mm. Hg for the males and 155/87 mm. Hg for the females. Of the females with diabetes 18 also had hypertension and 4 had demonstrable coronary artery disease. Six men with diabetes also

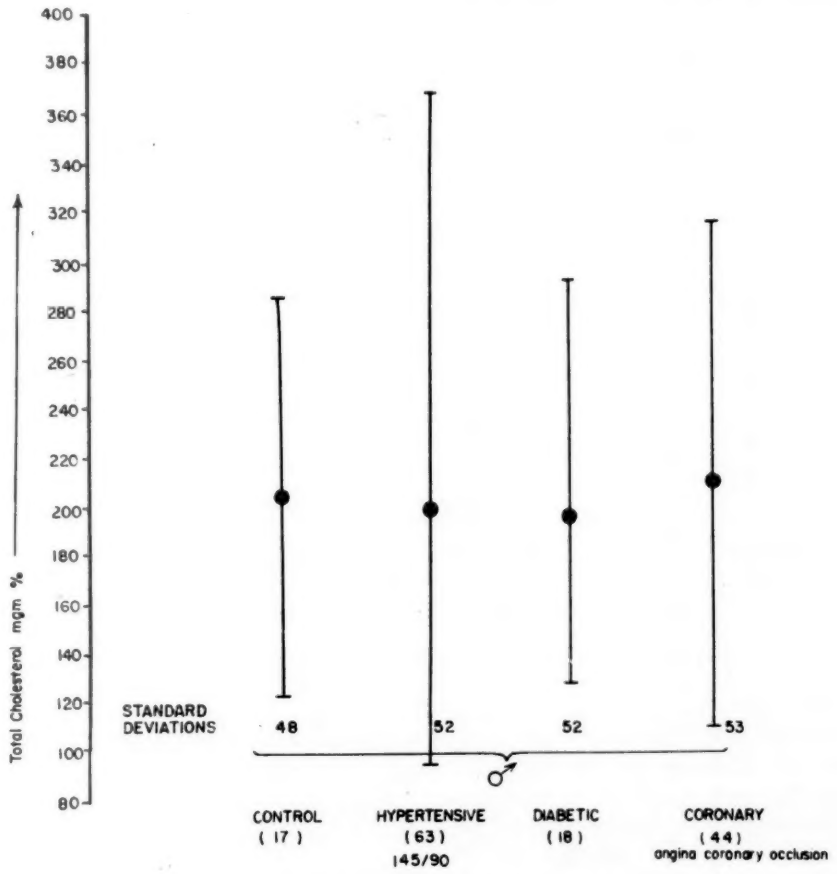

FIG. 1.—Total cholesterol findings in male subjects.

* The S_f 12-20 analyses were made through the cooperation of Martin Hanig, Ph.D. and Max Lauffer, Ph.D. of the University of Pittsburgh Department of Biophysics.

had hypertension and 3 of these had coronary artery disease.

In the 44 males and 30 females with coronary disease, the average ages were 59 and 60 respectively. The average weights were 157 and 149 pounds, and the mean systolic blood pressure of the males in this group was 140/85 and in the females 158/93 mm. Hg.

Results. The data obtained in this study are presented in Fig. 1 to 6. Fig. 1 indicates the total cholesterol findings in male subjects. In 17 normal subjects, the total cholesterol level varied from 122 to 281 mg. per 100 cc. with a mean value of 202 mg. per 100 cc. The mean value for 63 hypertensive subjects was 200 mg. per 100 cc. with a range of 90 to 366 mg. per 100 cc. In 18 diabetics the mean value was 197 mg. per 100 cc. with a range of 128 to 292 mg. per 100 cc. In 44 males suffering from coronary artery disease, the mean value was 210 mg. per 100 cc. with a range of 106 to 319 mg. per 100 cc.

Fig. 2 records the total cholesterol findings in female subjects. In 12 normal individuals the mean value was 200 mg. per 100 cc. with a range of 166 to 239 mg. per 100 cc. In 92 hypertensive females the mean value was 213 mg. per 100 cc. with a range of

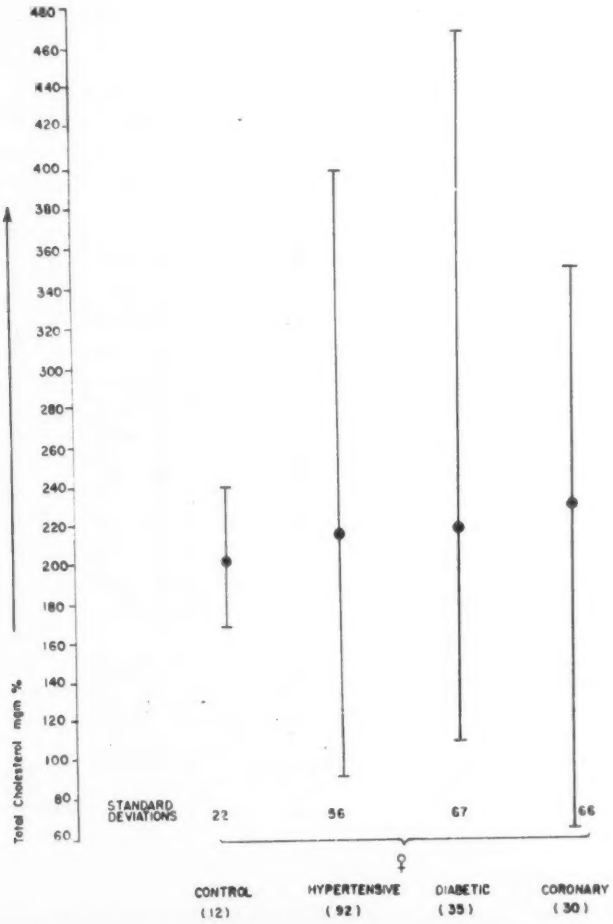

FIG. 2.—Total cholesterol findings in female subjects.

from 90 to 400. In 35 diabetic subjects the mean value was 218 mg. per 100 cc. with a range of 107 to 464 mg. per 100 cc. In 30 women suffering from coronary disease the mean value was 222 mg. per 100 cc. with a range of from 64 to 347 mg. per 100 cc.

The mean total cholesterol values for the data from both sexes revealed a mean for the normal subjects of 201 mg. per 100 cc., 208 mg. per 100 cc. for the hypertensives, 215 mg. per 100 cc. for the coronaries, and 208 mg. per 100 cc. for the diabetics.

There was no significant difference between the mean values for total cholesterol found in any of these groups. This was true when the data from the sexes was considered separately or when it was compared to data from normals or to data from the opposite sex with the same condition. The p value for the female diabetics compared to normals of the same sex was 0.25, well above the level of statistical significance; that is, p greater than 0.05.

Fig. 3 records the S_f 12-20 concentration in milligrams for the male subjects. The 17 normal Males have a mean value of 40 mg. per 100 cc. with a range of 18 to 76 mg. per 100 cc., the 61 hypertensives had a mean value of 50 mg. per 100 cc. with a range of 17 to 267. The 18 diabetic subjects had a mean value of 47 mg. per 100 cc. with a range of 17 to 95 mg. per 100 cc., and the 41 coronary subjects had a mean value of 42 mg. per 100 cc. with a range

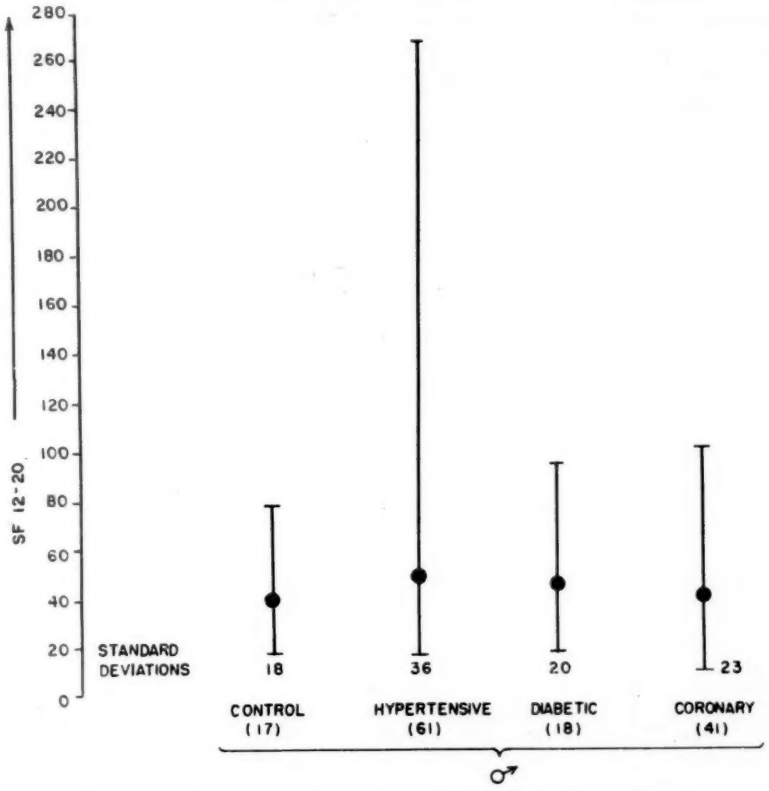

FIG. 3.—S_f 12-20 concentration in milligrams in male subjects.

of from 13 to 107. Data from females (Fig. 4) revealed in 12 normal subjects a mean value of 30 mg. per 100 cc. with a range of from 6 to 76 mg. per 100 cc. In 90 hypertensive females the mean value was 43 mg. per 100 cc. with a range of from 6 to 112 mg. per 100 cc. In 36 diabetic subjects the mean value was 54 mg. per 100 cc. with a range of from 20 to 94 mg. per 100 cc. Twenty-eight females with coronary disease revealed a mean value of 52 mg. per 100 cc. with a range of from 15 to 98 mg. per 100 cc.

The combined means for both sexes of S_f 12-20 values in each group revealed in the normals a mean of 35 mg. per 100 cc., in the hypertensives of 46 mg. per 100 cc., in those with coronary disease 47 mg. per 100 cc., and in the diabetic group a mean of 52 mg. per 100 cc.

There was no significant difference in the males between the mean of the S_f 12-20 concentration among the hypertensive, coronary, diabetic, and normal groups. The mean values of the females with hypertension, coronary disease,

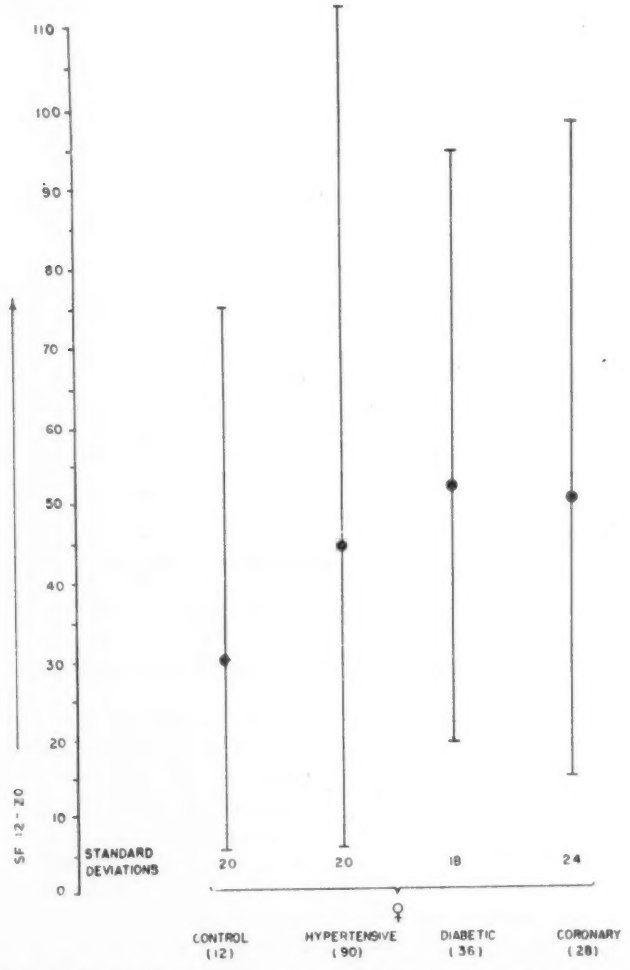

FIG. 4.–S_f 12-20 concentration in milligrams in female subjects.

and diabetes were significantly higher than with those of the normals, with a p value for the hypertensive females of 0.03, for the females with coronary disease of 0.02, and for the females with diabetes of 0.0005. It should also be noted that in the subjects with coronary disease the mean S_f 12-20 value with the males was statistically lower than that of the females, this being significant with a p value of 0.04. When the data from both sexes was combined the mean of the S_f 12-20 concentration of the hypertensive, coronary, and diabetic groups all significantly exceeded that of the normal group, demonstrating p values of 0.017 in the hypertensive group, 0.005 for the coronary group, and less than 0.0001 in the diabetic group.

Fig. 5 records the cholesterol-lipid phosphorus ratios obtaining in the males in this series. In 17 normal subjects a mean value of 20 was obtained with a range of from 14 to 27, in 62 hypertensive males the mean value was 19 with a range of from 11 to 30, in 18 diabetics the mean value was 17 with a range of from 12 to 22, and in 44 males with coronary disease the mean value was 20 with a range of from 11 to 32.

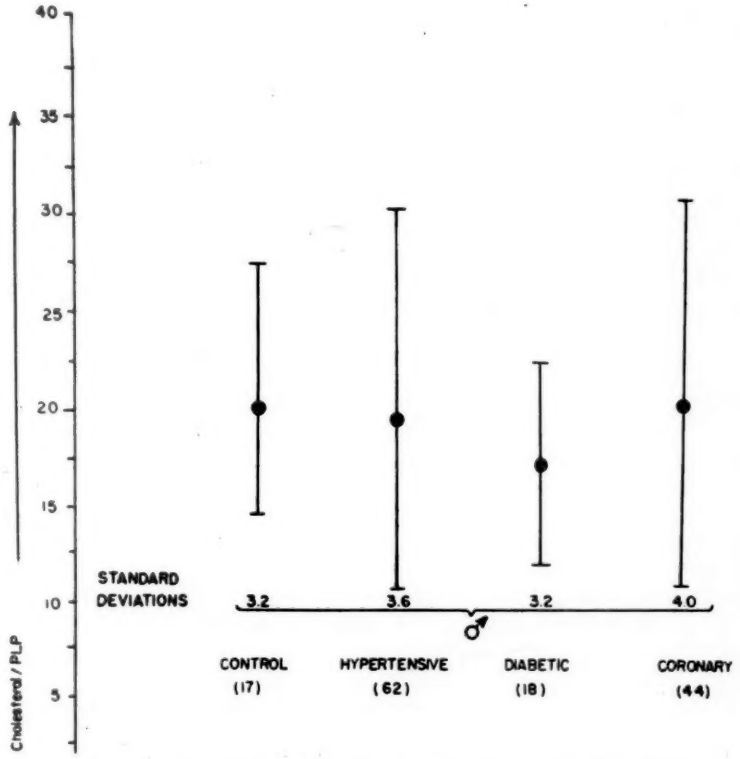

FIG. 5.—Cholesterol-lipid phosphorus ratios in male subjects.

Fig. 6 records the cholesterol-lipid phosphorus ratio in females. In 11 normal females the mean value was 21 with a range of 6 to 24. In 94 hypertensive females the mean value was 19 with a range of 10 to 39. In 36 diabetics the mean value was 18 with a range from 9 to 39. When the

cholesterol lipid phosphorus ratios for each sex were combined, the mean values for the normals were 21, for the hypertensives 19, for the diabetics 17, and for the coronary subjects 20. When the mean for each group for each sex was compared to that of the normal mean of the same sex, the only one that was statistically different in mean cholesterol lipid phosphorus ratio was that of the male diabetics. None of the means of either sex differed significantly from that of the opposite sex in the same group. When the data for both sexes were combined, both the means for the hypertensive and for the diabetics differed significantly from the mean of the normal group. In the case of the hypertensives, this demonstrated a p value in the combined sexes of 0.026 and in the diabetics a p value of 0.004.

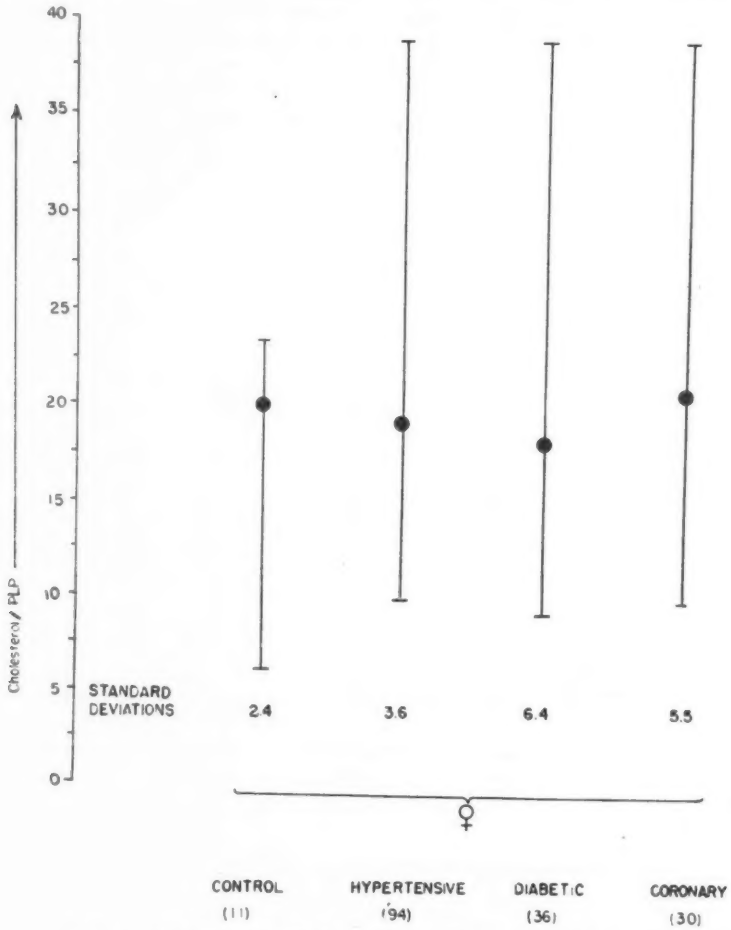

FIG. 6.—Cholesterol-lipid phosphorus ratios in female subjects.

In the 18 female diabetics who also had hypertension, the mean total cholesterol value was 225 mg. per 100 cc., the S_f 12-20 concentration was 47 mg. per 100 cc., and the cholesterol-lipid phosphorus ratio 19.0. In the 4 females with diabetes, hypertension,

and coronary disease the mean total cholesterol was 247 mg. per 100 cc. and the S_f 12-20 concentration 53 mg. per 100 cc. Lipid phosphorus determinations were not available on all of these 4 subjects. None of these mean values in women with more than one disorder are significantly different from the means of the normal subjects or those with diabetes alone.

Only 6 male subjects had both diabetes and hypertension. The mean total cholesterol value in this group was 216 mg. per 100 cc., the S_f 12-20 concentration 52 mg. per 100 cc., and the cholesterol-lipid phosphorus ratio 18.1. In the 3 men with diabetes, hypertension, and coronary disease although the mean total cholesterol was only 249 mg. per 100 cc. and the cholesterol-lipid phosphorus ratio 20.7, the mean S_f 12-20 concentration was elevated to 63 mg. per 100 cc. Although interesting, this is not a significant elevation because of the small number of subjects.

Discussion. The data presented indicates that in the subjects of this study there was no significant difference in the mean total cholesterol values found in either sex in any of these groups. The wide variation in total cholesterol value found in both normal subjects and patients with hypertension, diabetes, and coronary disease is a common clinical finding. It is quite likely that a larger series of subjects would permit the demonstration of significantly differing mean values among these groups. However, because of the wide range of values obtained in the several groups, the diagnostic value of the total cholesterol determination in an individual patient leaves much to be desired.

The data obtained on the S_f 12-20 concentration failed to indicate any significant differences between the mean values in the males in the normal and patient groups. However, the mean values for females with hypertension, coronary disease and especially diabetes were significantly higher than those found for normal women. However, again the wide range of values obtaining in all groups sharply limits the diagnostic value of this procedure in a particular patient.

Similarly, although the mean cholesterol-lipid phosphorus ratio differed significantly in male diabetics from normal males, there was again considerable overlapping in the range of values obtained.

In view of the wide range of values for total cholesterol, S_f 12-20, and cholesterol-lipid phosphorus ratio found in both normal subjects and patients with hypertension, coronary disease, and diabetes, it is suggested that the values obtaining in any individual be interpreted quite cautiously until more accurate information on the significance of these findings in the pathogenesis of vascular disease is available.

Summary. 1. Total cholesterol values, S_f 12-20 concentration, and cholesterol-lipid phosphorus ratios are reported in a series of normal subjects and patients with hypertension, diabetes, and coronary artery disease.

2. No significant differences were found in the mean total cholesterol values among any of these groups.

3. The mean value for S_f 12-20 concentration in females with hypertension, coronary disease, and especially diabetes were significantly higher than those found in normal women.

4. The mean cholesterol-lipid phosphorus ratio in male diabetics was significantly lower than that of normal males.

5. In the small number of subjects in this series the presence of either or both hypertension or coronary artery disease in patients with diabetes failed to be accompanied by significant increases in the mean total cholesterol or S_f 12-20 concentration

above those obtained in diabetics without these complications.

6. These findings emphasize the wide range of values for total cholesterol, S_f 12-20 and cholesterol-lipid phosphorus ratio in normal subjects and those with hypertension, diabetes, or coronary disease and demand that the significance of a value found in any individual be interpreted quite cautiously until more accurate information on the signifiance of these findings in the pathogenesis of vascular disease is available.

The authors wish to express their gratitude to Miss Agatha DeLacio and Mrs. Grace Rhodes for technical assistance and Mr. Carroll Weil for aid in the statistical analysis.

REFERENCES

1. **Fiske, C. H.,** and **SubbaRow, Y.**: J. Biol. Chem. **66,** 375, 1925.
2. **Foldes, F. R.,** and **Wilson, B. C.**: Anal. Chem. **22,** 1210, 1950.
3. **Gofman, J. W., Lindgren, F. T.,** and **Elliott, H.**: J. Biol. Chem. **179,** 973, 1949.
4. **Gofman, J. W., Lindgren, F., Elliott, H.,** and **Mantz, W.**: Science **111,** 166, 1950.
5. **Hanig, M.,** and **Lauffer, Max**: Presented June 8, 1952, at the Chicago meeting of the American Diabetes Association.
6. **Keys, Ancel**: J. Am. Med. Assn. **147,** 1514, 1951.
7. **Mann, G. V., McNally, A.,** and **Andrus, S. B.**: Presented April 18, 1952, at the Cleveland, Ohio, meeting of the American Heart Association.

SERUM QUINIDINE CONCENTRATION IN CONGESTIVE HEART FAILURE

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The use of quinidine to convert auricular fibrillation to normal sinus rhythm has been the subject of a number of articles within the past few years[4,5,7,8,10,11]. Although treatment directed toward the relief of concomitant congestive heart failure generally preceded the administration of quinidine, a substantial number of patients developed one or more manifestations of quinidine toxicity. In a group of 6 patients recently reported[9] who developed ventricular tachycardia following the use of quinidine, 4 had recently or incompletely recovered from congestive failure. It, therefore, seemed important to determine whether the levels of quinidine achieved in the blood following the oral administration of quinidine sulfate in patients with congestive failure differed from those obtaining in normal persons.

Methods. The subjects were 11 patients, with congestive heart failure manifested by pulmonary congestion, enlargement of the liver, edema, elevated venous pressure, and prolonged circulation time. All had cardiac enlargement. Etiologic factors included rheumatic, hypertensive, arteriosclerotic and pulmonary heart disease as well as one patient with constrictive pericarditis.

Control observations were made on 16 patients who had no evidence of cardiac, renal, or hepatic disease.

One patient was studied twice while in congestive failure, and 6 were restudied after all evidence of failure had disappeared.

Quinidine sulfate was administered orally in 3 doses of 0.4 gm. each at 2-hour intervals. Samples of venous blood for quinidine analysis were withdrawn at varying intervals between 2 and 12 hours after the last dose of quinidine. Following clot retraction the serum was separated by centrifugalization and analyzed by the method of Brodie and his co-workers[1] as modified by Linenthal[6]. This method utilizes the extraction of quinidine by ethylene dichloride and has been found as satisfactory as the protein precipitation procedure[3].

The details of the method are outlined: 30 cc. glass-stoppered bottles were used throughout. Ten cubic centimeters of ethylene dichloride were transferred by suction to the bottles. The toxicity of the substance makes it imperative that a mechanical suction device be employed for pipetting. One cubic centimeter of normal serum was added to one bottle, one cubic centimeter of normal serum and one cubic centimeter of quinidine solution containing 5 mg. per liter to another, and one cubic centimeter of each unknown serum to the remaining bottles. One cubic centimeter of 10% sodium hydroxide was added to each bottle, and the bottles were agitated in a mechanical shaker for 10 minutes. The contents were transferred to 15 cc. centrifuge tubes and spun for 5 minutes at 2500 revolutions per minute. The top layers were removed by suction and discarded. An aliquot portion of 4 cc. was transferred by suction from each tube to separate bottles, to each of which 15 cc. of tenth normal sulfuric acid had already been added. These bottles were shaken for 10 minutes and the contents transferred to 50 cc. tubes and spun for 5 minutes at 1500 revolutions per minute. The top layers were removed by suction and discarded. Samples from the centrifuge tubes

were transferred to special cuvettes for measurement of fluorescence.

A Beckman spectrophotometer adapted for photofluorometry was used. The differences in fluorescence between normal serum, serum plus a known concentration of quinidine, and unknown sera were measured directly and quinidine levels estimated accordingly.

Results. The highest concentration of quinidine in the serum was achieved in 2 to 4 hours after the last dose in all but one of the 34 studies on both control subjects and those with or after recovery from congestive failure. The actual peak level observed varied from 2.3 to 8.4 mg. per liter with no conspicuous difference between control subjects and patients. Since observations were made at 1 to 2-hour intervals, it is probable that the actual maximum serum concentration was often higher between determinations inasmuch as a fall of 20% occurred in one hour in a number of instances (Tables 1 and 2).

Analysis of the values obtained at 2-hour intervals in 6 of the control subjects suggested that the disappearance of quinidine from the serum follows an exponential decay curve and that the rate of disappearance was essentially constant for any one individual, but varied widely in different subjects. This is in agreement with the observations of Delevett and Poindexter[2].

The serum levels at the end of 12

TABLE 1.—QUINIDINE SERUM LEVELS IN CONTROL SUBJECTS

	Hours After Last Dose							
Case	*2*	*3*	*4*	*6*	*8*	*10*	*12*	*Peak*
1	3.3	3.5	2.6				0.3	3.5
2	3.4	3.3	2.9				1.0	3.4
3	2.3	3.5	3.0				1.1	3.5
4	3.9	4.4					1.8	4.4
5	8.4	8.2	7.4				3.9	8.4
6	2.9	2.6	2.6				0.9	2.9
7	5.7	5.5	5.0				2.0	5.7
8	6.8	5.8					1.8	6.8
9	1.6	2.9	2.8				0.5	2.9
10	5.6		5.9				0.8	5.9
11	1.7		2.2	2.8	1.6	1.2	1.0	2.8
12	4.8		3.7	2.6	1.6	1.3	1.1	4.8
13	4.0		3.7	2.2	1.1	0.6	0.5	4.0
14	6.7		7.4	7.0	5.7	4.9	3.9	7.4
15	2.3		1.6	1.3	0.6	0.5	0.3	2.3
16	4.1		4.4	2.9	1.9	1.6	1.1	4.4
Avg.	4.15	4.4	3.59	3.13	2.1	1.7	1.37	4.57

TABLE 2.—QUINIDINE SERUM LEVELS IN PATIENTS WITH CONGESTIVE HEART FAILURE

	Decompensated Hours After Last Dose					*Compensated Hours After Last Dose*					
	2	*3*	*4*	*12*	*Peak*	*2*	*3*	*4*	*12*	*Peak*	*Weight Loss*
1a	5.4	6.2	6.0	5.5	6.2						
1b	5.8	6.8		4.4	6.8						
2	4.1	4.8		3.7	4.8						
3	4.4	3.2		0.3	4.4						
4	7.6	6.9		4.9	7.6						
5	3.1	3.6	3.4	1.5	3.6						
6	2.2	2.4	3.0	1.8	3.0	4.1	3.7	2.2	0.0	4.1	27
7	2.6	3.7	4.3	2.7	4.3	2.9		2.4	0.8	2.9	12
8	4.2	4.1	3.8	2.2	4.2	2.5	2.4	1.8	0.6	2.5	30
9	4.7	3.0	3.0	2.0	4.7	2.8	3.3	2.8	1.7	3.3	45
10	3.1	3.3	3.0	2.1	3.3	3.8	3.8	3.5	2.5	3.8	11
11	3.0	3.9	3.2	1.4	3.9	3.5	4.7	3.3	1.5	4.7	31
Avg.	4.18	4.32	3.71	2.7	4.73	3.27	3.58	2.67	1.18	3.55	

hours were moderately reduced in patients with congestive failure and markedly reduced in the other groups with the exception of 2 of the normal subjects. In the patients with failure 57% of the peak level remained as contrasted with only 30% in the control subjects (Fig 1).

Summary and Conclusions. In general the patients with congestive failure showed higher serum levels at the end of 12 hours than did the control subjects, although the time of appearance in the blood stream and the peak levels did not differ significantly from the latter. This suggests that the rate of disappearance was slower in the patients with failure.

The recognition that patients with failure will show considerable delay in lowering of the blood quinidine level is of great importance in the continued administration of this drug. Unusually high serum levels and serious toxicity can occur in these patients. If this hazard is to be avoided, patients with failure should be given quinidine with caution and continued administration should be controlled by serum quinidine determinations.

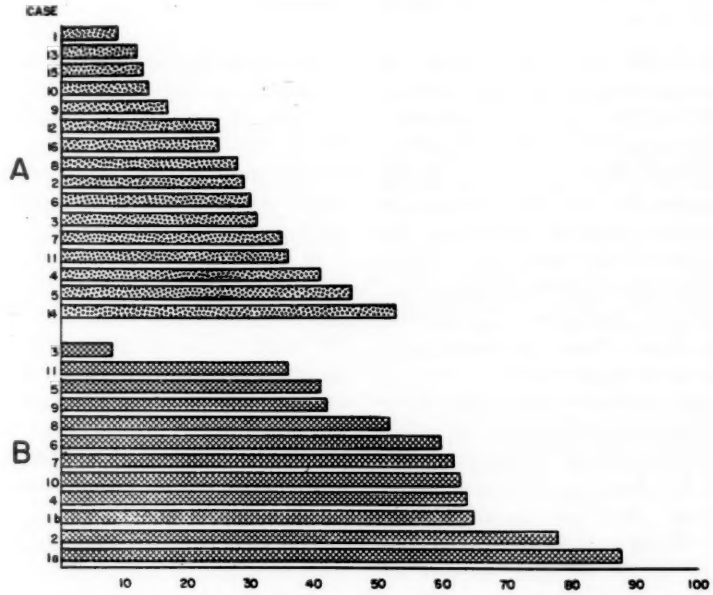

FIG. 1.—Per cent of peak quinidine level remaining twelve hours after last dose. A. Normal subjects. B. Patients in congestive heart failure.

REFERENCES

1. **Brodie, B., Udenfriend, C., Dill, W.,** and **Downing, G.:** J. Biol. Chem., **168,** 311, 1947.
2. **Delevett, A. F.,** and **Poindexter, C. A.:** Am. Heart J., **32,** 697, 1946.
3. **Edgar, A. L.,** and **Sokolow, M.:** J. Lab. & Clin. Med., **36,** 478, 1950.
4. **Holzman, D.,** and **Brown, M. G.:** Am. J. Med. Sci., **222,** 644, 1951.
5. **Houston, A. B.,** and **Perry, W. F.:** Canad. Med. J., **63,** 56, 1950.
6. **Linenthal, A.:** Personal Communication.
7. **Sokolow, M.:** Am. Heart J., **43,** 771, 1951.
8. **Sokolow, M.,** and **Edgar, A. L.:** Circulation, **1,** 576, 1950.
9. **Wetherbee, D. G., Holzman, D.,** and **Brown, M. G.:** Am. Heart J., **43,** 89, 1952.
10. **Yount, E. H.,** and **Rosenblum, H.:** South. Med. J., **43,** 324, 1950.
11. **Yount, E. H., Rosenblum, H.,** and **McMillan, R. L.:** Arch. Int. Med., **89,** 63, 1952.

STUDY OF THE MERCURIAL DIURETIC, DICURIN PROCAINE (MERETHOXYLLINE PROCAINE) BY SUBCUTANEOUS INJECTION*

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MANY attempts have been made to develop a non-irritant organic mercurial diuretic which may be administered subcutaneously. The introduction of a theophylline or a thiol group into the molecule has been found to reduce toxicity without lessening the diuretic effect of the mercury[1]. Thus meralluride sodium (Mercuhydrin) and mercaptomerin sodium (Thiomerin, Diucardyn) have been recommended for subcutaneous injection[1,4].

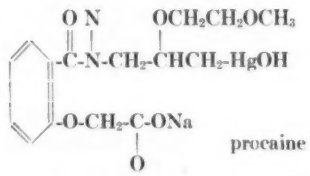
Studies in animals and man have suggested that the subcutaneous injection of mercaptomerin sodium is accompanied by less local tissue reaction than from other mercurial diuretic agents. Enselberg and Simmons[2] stated in February 1950, in their report of 3314 subcutaneous injections of Thiomerin in 205 patients, that they had encountered no local reactions since 1948.

Recently, in a critical review of the literature and their own studies, Gold and co-workers[3] have found that more than 25% of patients develop "troublesome" local reactions after subcutaneous injections of Thiomerin. They state that while Thiomerin is a potent and substantially safe diuretic agent, it is not sufficiently free of local reactions to meet the need for a mercurial diuretic for subcutaneous injection.

Thus it would seem advisable to study the effects of other mercurial compounds in an attempt to find a safer, more effective mercurial diuretic for subcutaneous injection.

A new mercurial compound. merethoxylline procaine with theophylline, has recently been made available to us for study. The preparation is a coordination complex of procaine with the sodium salt of the mercury compound. The formula is as follows: sodium *o*-(N–hydroxy-mercuri–methoxyethoxy)-propyl-carbamyl) phenoxyacetate procaine.

The structural formula is as follows:



The compound is known to us as Dicurin Procaine, and will henceforth be referred to as such in this study.

* Supported in part by Grant-in-Aid, Eli Lilly and Company, Indianapolis, Indiana.

The mercurial compound used in Dicurin Procaine appears to be unique in that it forms a chemical combination with the procaine base. The molecular weight of the entire molecule is 785, and the percentage of mercury is 25.43 expressed as percentage of molecular weight.

Studies in animals by other investigators[7] have shown the diuretic properties and systemic toxicity of Dicurin Procaine to be similar to mercaptomerin sodium. The LD_{50} for Dicurin Procaine as tested in mice by intravenous injection, expressed as milligrams of mercury per kilogram, is 24.45 ± 3.9; for mercaptomerin sodium 22.4 ± 4.00. Subcutaneously in the rat, the LD_{50} is 12.56 ± 0.56 as compared with 13.8 ± 0.64 for mercaptomerin sodium. These slight differences are not considered significant.

During the investigation of the compound we have accumulated data which seem worthy of presentation at this time.

Methods. a) The effects of Dicurin Procaine and mercaptomerin sodium on water and electrolyte excretion in edematous patients:

Four edematous patients, who had chronic dependent edema of cardiac origin, and who had received no mercurial diuretics, were selected for study. The patients were hospitalized and placed on cardiac diets containing less than 800 mg. of sodium daily. Water was permitted as desired. A control 24-hour urine specimen was obtained on the third day of hospitalization. A 1 or 2 cc. injection of Dicurin Procaine was then given subcutaneously, and hourly urine specimens were collected for the next 11 hours, and a final 13-hour pooled specimen was then collected.

The content of sodium, potassium and chloride was determined in the 24-hour urine specimen. The patients were then permitted to reaccumulate the edema and return to their original weights. A similar procedure was then carried out following the subcutaneous injection of 1 or 2 cc. of mercaptomerin sodium.

b) Effects of Dicurin Procaine in conditions characterized by edema:

All patients studied were edematous. The edema was due to chronic congestive heart failure of long duration, or was associated with hepatic cirrhosis. All had been in regular clinic attendance for periods ranging from 6 months to 10 years. The patients with cardiac diseases had required frequent injections of mercurial diuretics in addition to the usual use of digitalis and intermittent administration of ammonium chloride or a cation exchange resin. The patients with portal cirrhosis had received a high protein diet with vitamin supplements.

The mercurial diuretic administered during the preceding few months was mercaptomerin sodium, 1 or 2 cc. intramuscularly as needed to maintain the patient ambulatory and fairly edema free. The usual weekly requirement of this mercurial was determined for each patient. All patients had been advised to adhere to a low sodium diet, but cooperation in this respect was probably only fair.

The only change made in the management of these patients was the substitution of Dicurin Procaine, 1 or 2 cc. administered *subcutaneously*, for the previously administered mercaptomerin sodium. The usual dosage of ammonium chloride or the cation exchange resin was continued throughout the study.

The patients remained ambulatory throughout the study. Initially a complete blood count, urinalysis, serum urea nitrogen and serum chloride determinations were performed. The studies of serum urea nitrogen and serum chloride, as well as a urinalysis were performed at bi-weekly intervals. Serum sodium determinations were performed as indicated by hypochloremia or evidence of the so-called "low salt syndrome." The complete blood count was repeated at the conclusion of the study.

All patients were seen by the authors at each clinic visit and all injections were given by them. The injections were administered subcutaneously with a 23 or 25 gauge needle, usually in the region overlying the deltoid muscle. Body weight was recorded, the site of the previous injection examined, and the patients were questioned as to possible local or systemic reactions.

c) Further studies on patients exhibiting ecchymosis following Dicurin Procaine:

The patients who developed ecchymotic areas at the site of injection following the Dicurin Procaine were subjected to further studies in an attempt to determine if any predisposing condition was present. Hematological studies consisted of determination of bleeding time, clotting time, clot retraction,

platelet counts, prothrombin time, and plasma ascorbic acid levels. Tourniquet tests were performed. Liver function tests consisting of cephalin flocculation, thymol turbidity, total protein with albumin/globulin ratio and cholesterol/cholesterol ester ratio were also performed.

Subcutaneous injections, at weekly intervals, of saline, mercaptomerin sodium and a repeated injection of Dicurin Procaine were then administered to these patients. Intramuscular injection of the same compounds were then made to determine the constancy of local reactions.

The analytical determinations were performed by the following methods: serum and urine sodium, and potassium determinations were performed on a Weichselbaum Varney Universal Spectrophotometer; serum and urine chloride, by Van Slyke and Hiller's modification of Sendroy's method; bleeding time by Duke's method; clotting time by Lee and White method; clot retraction by Kolmer method; prothrombin time by Quick's quantitative method, and ascorbic acid by the method of Farmer and Abt.

Results. Table 1 shows the mean hourly urine output following the subcutaneous injection of similar quantities of Dicurin Procaine and mercaptomerin sodium. The time relations were similar in all patients and did not seem to vary with the size of the diuresis. The diuresis was accompanied by an increased excretion of sodium, potassium and chloride as shown in Table 1. Unrecognized variation in

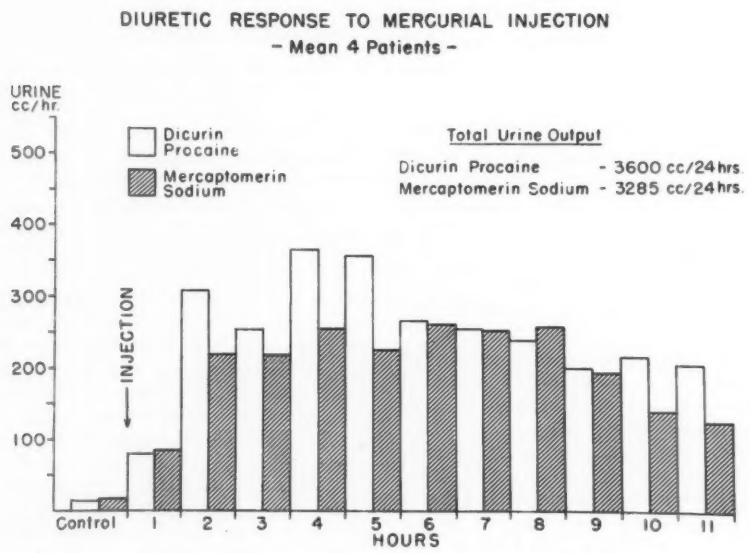


FIG. 1.—Diuretic Response to Mercurial Injection.

TABLE 1.—EFFECTS OF SUBCUTANEOUS INJECTIONS OF DICURIN PROCAINE AND MERCAPTOMERIN SODIUM ON URINE ELECTROLYTE EXCRETION

	Sodium mEq./24 hrs.			*Potassium mEq./24 hrs.*			*Chlorides mEq./24 hrs.*		
Patient	*Control*	*Dicurin Procaine*	*Mercaptomerin Sodium*	*Control*	*Dicurin Procaine*	*Mercaptomerin Sodium*	*Control*	*Dicurin Procaine*	*Mercaptomerin Sodium*
Beavin	0.62	198.1	257.6	4.3	57.1	61.3	9.6	254.5	335.7
Bethel	69.7	248.0	228.0	26.6	56.5	76.0	62.2	209.6	230.7
Hicks	42.2	521.5	457.6	12.9	24.6	22.2	29.4	523.2	430.8
Edlin	34.5	451.3	356.4	24.7	12.3	24.5	16.3	439.7	344.6

diet or fluid intake may have influenced the results so that the differences noted in sodium, potassium and chloride excretion may not be significant. They suggest a similar type of mercurial diuresis following the injection of the 2 compounds.

The data concerning the 69 clinical patients treated by subcutaneous administration of Dicurin Procaine are summarized in Table 2. It may be noted that a reaction occurred in about one-fifth of the patients. Some type of local tissue reaction accompanied 8.15% of the injections.

Local reactions noted consisted of pain lasting more than 8 hours at the site of injection, subcutaneous nodule formation and areas of ecchymosis, as shown in Table 3. In general the reactions were not severe, and in no case did the patient demand the discontinuance of the compound.

Six patients (8.7% of the total patients) developed areas of ecchymosis. These usually consisted of painless areas of discoloration ranging from 3 to 9 cm. in diameter which developed from 6 to 8 hours after the injection. It may be significant that all cases of ecchymosis occurred in females over the age of 64 years. Table 4 shows the result of the blood studies in these patients. All liver function tests studied in the group were normal. Table 5 shows the result of intra-

TABLE 2.—LOCAL REACTIONS FOLLOWING 540 SUBCUTANEOUS INJECTIONS OF DICURIN PROCAINE IN 69 PATIENTS CLASSIFIED BY DISEASE AND SEX

Etiology	*Sex*	*Number of Patients*	*Number of Injections*	*Reactions*	
				Patients	*Injections*
CHRONIC CONGESTIVE HEART FAILURE					
Arteriosclerotic	M	13	100	2	2
"	F	5	34	1	5
Hypertensive	M	17	145	4	14
"	F	14	64	4	16
Rheumatic	M	0	0	0	0
"	F	4	52	0	0
Syphilitic	M	5	42	0	0
"	F	3	19	2	6
Undiagnosed	M	3	35	0	0
"	F	2	12	1	1
CIRRHOSIS OF THE LIVER					
	M	3	37	0	0
	F	0	0	0	0
Totals		69	540	14	44

Average Weekly Mercurial Requirement:
Control Period (Thiomerin, I.M.) 2.76 cc./week
Treatment Period (Dicurin, Sub Q) 2.82 cc./week
% Patients with reactions 20.30%
% Injections with reactions 8.15%

TABLE 3.—LOCAL REACTIONS FOLLOWING 540 SUBCUTANEOUS INJECTIONS OF DICURIN PROCAINE IN 69 PATIENTS

Type	*Number of Patients*	*% of Patients*	*Number of Injections*	*% of Injections*
Pain	3	4.35	8	1.48
Nodule	9	13.04	25	4.63
Ecchymosis	6	8.70	16	2.96

(Some patients exhibited more than one type of reaction)

muscular and subcutaneous injection of saline, mercaptomerin sodium and Dicurin Procaine in these patients.

There was no evidence of toxicity as measured by repeated blood studies and urinalyses in the entire group of 69 patients. No patient exhibited signs of systemic toxicity or hypersensitivity reactions. Three patients died during the period of study of their underlying cardiac disease. One patient developed oliguria, at which time the serum sodium was 123.8 mEq./liter and serum chloride was 79 mEq./liter. He responded immediately to the intravenous administration of 5% sodium chloride solution.

Comment. The subcutaneous injection of a mercurial diuretic possesses the major advantage of an increased ease of self-administration, with considerable saving of time and expense. The well-known protoplasmic toxicity of the mercury ion makes it unlikely that an organic mercurial diuretic will be developed that is entirely devoid of local irritant action. This fact, and the difficulty of adequate supervision by the physician make it probable that the majority of mercurial diuretic injections will continue to be administered intramuscularly. However, it is a definite advantage for a particular mercurial compound to be accompanied by a minimal local tissue irritant action when administered subcutaneously by choice or through technical error.

TABLE 4.—BLOOD STUDIES ON SIX PATIENTS EXHIBITING AREAS OF ECCHYMOSIS FOLLOWING SUBCUTANEOUS INJECTIONS OF DICURIN PROCAINE

	Name of Patient					
	Hieatt	*Hibbs*	*Plantholt*	*Lucas*	*Peters*	*Curry*
Prothrombin time	Over 100%	91%	96%	Over 100%	80%	74%
Bleeding time	35″	28″	32″	30″	70″	25″
Clotting time	9′48″	13′10″	11′00″	17′55″	9′35″	13′15″
Platelet count	391,000	407,920	416,000	300,160	119,080	457,000
Vitamin C mg/100cc.	1.4	0.9	1.3	0.9	0.9	0.8
Tourniquet test	normal	+++	+	normal	+++	0 or +
Clot retraction	complete	complete	complete	complete	poor	complete

TABLE 5.—SIX PATIENTS EXHIBITING ECCHYMOSIS FOLLOWING MERCURIAL DIURETICS

	Dicurin Procaine			*Mercaptomerin Sodium*		*Saline*
Patient	*Subcutaneous Injection*	*Repeat Injection Subcutaneously*	*Intramuscular Injection*	*Subcutaneous Injection*	*Intramuscular Injection*	*Subcutaneous Injection*
Hieatt	yes	no	no	yes	no	no
Peters	yes	yes	yes	yes	no	yes
Curry	yes	no	no	yes	yes	no
Lucas	yes	no	no	no	no	no
Hibbs	yes	no	no	no	no	no
Plantholt	yes	no	no	no	no	no

The compound herein reported appears to be satisfactory for subcutaneous injection. The incidence of local reactions occurring in 20.3% of the patients, following 8.15% of injections, compares favorably with the observations of Gold and co-workers[3] of local tissue reactions in 44% of a series of patients following 27% of all subcutaneous injections with Thiomerin.

The development of areas of painless ecchymosis have been reported following the injection of several mercurial compounds[1,2,3]. Our data suggest that the local action of the organic mercury ion itself is the major factor. Studies of blood and blood coagulation elements revealed no consistent abnormalities. Prolongation of clotting time was noted in one patient, and reduction of blood platelets with poor clot retraction occurred in one other patient. The most frequent abnormality was a positive tourniquet test. This finding in an elderly female patient would seem to render inadvisable the subcutaneous injection of Dicurin Procaine. The data would indicate that intramuscular administration would be the method of choice in such patients. The areas of ecchymosis while unsightly were never accompanied by slough or other serious consequences.

No evidence of systematic toxicity or hypersensitivity was noted in these patients. Five of the patients were intolerant to other mercurial compounds yet were able to tolerate Dicurin Procaine. It is not unusual for a patient to tolerate one particular mercurial compound while being intolerant of related compounds. It seems likely that further experience will reveal individuals intolerant to Dicurin Procaine.

The diuretic effectivenes of a 2 cc. injection of Dicurin Procaine and mercaptomerin sodium seems to be very similar. It is of interest to note that 2 cc. of Dicurin Procaine contains 78 mg. of mercury, as compared to 80 mg. of mercury contained in 2 cc. of mercaptomerin sodium. It is unfortunate that the comparison made utilized the intramuscular injection of mercaptomerin sodium, but strong patient preference necessitated this mode of administration. It should be noted that the Dicurin Procaine was well accepted by the patients.

Dicurin Procaine was stable throughout the study as determined by diuretic response and lack of change in color or turbidity of the solution. This is an advantage in that wastage is reduced compared to compounds such as mercaptomerin sodium which must be diluted prior to administration and are then stable only a limited period.

Summary and Conclusions. Studies are presented of a new organic mercurial compound, following subcutaneous injection in human patients. The compound, merethoxylline procaine with theophylline, (Dicurin Procaine) appears to be unique in that the mercurial compound forms a chemical combination with the procaine base. Our results may be summarized as follows:

1) Diuretic efficiency of Dicurin Procaine resembles that of mercaptomerin sodium.
2) Sixty-nine patients received 540 subcutaneous injections of the new mercurial compound. Local reactions occurred in 20.3% of the patients following 8.15% of the injections. The local reactions were not of sufficient severity to limit the use of the drug.
3) No systemic or hypersensitivity reactions were noted in the treated patients.
4) Ecchymosis was found only in female patients over the age of 64 years. It appears to be due to the local action of the mercury ion on the capillary vessels.

5) Dicurin Procaine is a satisfactory mercurial compound for subcutaneous use. However, the use of the compound is occasionally accompanied by undesirable local reactions.

ADDENDUM: Since this manuscript was submitted for publication, 428 additional injections of the compound have been administered subcutaneously with very similar results. Care should be taken to insure subcutaneous administration as ecchymosis and pain frequently follow the intracutaneous injection of mercurial compounds.

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REFERENCES

1. **Batterman, R. C., Unterman, D.,** and **DeGratt, A. C.**: J. Am. Med. Assn., **140,** 1268, 1949.
2. **Enselberg, C. D.,** and **Simmons, H. G.**: Am. J. Med. Sci., **219,** 139, 1950.
3. **Gold, H., Greiner, T., Mathes, S. B., Marsh, R. R., Warshaw, L. J., Modell, W., Kwit, N. T., Otto, H. L., Garb, S., Bakst, H.,** and **Kramer, M. L.**: Am. J. Med. Sci., **223,** 618, 1952.
4. **Gold, H., Kwit, N. T., Modell, W., Hanlon, L. W., Kramer, M., Greenburg, S., Otto, H. L., Catlove, E. W., Benton, J. G., Pearlmutter, M.,** and **Zahm, W.**: Am. J. Med., 3, 665, 1947.
5. **Kohlstaedt, K. G.**: Personal Communication.
6. **Ray, C. T.,** and **Burch, G. E.**: Circulation, **3,** 926, 1951.
7. **Robbins, E. B., Anderson, R. C.,** and **Harris, P. N.**: To be published.

INFECTIOUS HEPATITIS IN PREGNANCY*

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INFORMED obstetricians and internists regard the combination of pregnancy and infectious hepatitis as extremely rare[22,33,46]. There is little in the commonly available texts that will assist the physician when he encounters this combination[3,10,37]. With no previous record of hepatitis in pregnancy, this department cared for 4 such patients within the June-December period of 1951. No additional cases have been treated subsequently, although approximately 200 new patients register for care each month. This experience together with the paucity of information in current literature, has suggested a study and review the results of which are here briefly presented.

HISTORICAL. Infectious hepatitis is a relatively new term arising from experience in World War II. Previously, its mild form had been called catarrhal jaundice. As recently as 10 years ago, medical students were still being given their choice of 6 theories of its etiology. Catarrhal jaundice was regarded as being about as significant as the common cold when it complicated pregnancy.

We now know that the most severe variety of infectious hepatitis is associated with acute yellow atrophy of the liver[27]. This descriptive pathological term had heretofore been accorded the status of a disease. Despite the fact that the original reports of Thierfelder, Quincke and Kent[37] were of epidemics involving both men and non-pregnant women as well as pregnant women, it was listed as a toxemia of pregnancy. Further, no attempt to differentiate liver necrosis resulting from chemical intoxication was made when the patient was pregnant.

Pernicious vomiting of pregnancy is described in obstetrical texts with emphasis on liver damage and is usually illustrated by photomicrographs showing liver necrosis. Since the advent of dietary, electrolytic and psychotherapeutic measures, this condition has become extremely rare. The patient who is pregnant and who is suffering from vomiting and jaundice presents a clinical picture that is identical with that of moderately severe infectious hepatitis in the non-pregnant patient. It is conceivable that in the past cases described as ones of pernicious vomiting were actually a moderately severe hepatitis.

EPIDEMIOLOGY. Of viral origin, infectious hepatitis is a common disease. The usual manner of infection is the oral-intestinal-fecal route, with water an important source of infection. Dissemination is easy in case of gross pollution, and the ordinary devices to control pollution are ineffective against this virus[7]. It can also be transmitted by transfusion of blood and plasma. It is then clinically like homologous serum jaudice, from which it differs immunologically, and in a shorter incubation period; and in the fact that the virus of homologous serum jaundice has not been found in the stool. Except for albumin and globulin, which are

* The opinions expressed are those of the author and do not necessarily reflect those of the Navy Department.

processed sufficiently, every blood fraction has been the proven source of viral hepatitis[26]. Improperly sterilized equipment is a source of cross-infection. Since the entire population is susceptible, every obstetrician should be alert to the diagnosis of hepatitis. This is emphasized by the 35% incidence of serological and other evidence of infection in a group of inoculated volunteers without clinical symptoms, especially jaundice[15]. Chronic hepatitis and the carrier state are not unusual[38].

Since the obstetrician is a major user of whole blood in the treatment of anemias of pregnancy and obstetrical emergencies, he should be aware of the patient reported by Neefe[32]. This professional donor was found to be the source of hepatitis in several of his recipients, and yet he gave no history of infection. Study of this carrier revealed chronic hepatitis with fibrosis. There is no method available for sterilization of whole blood, and even the recently improved irradiation technique for sterilizing plasma has not proved satisfactory in mass production. The mere elimination of donors with a history of jaundice is inadequate.

INCIDENCE. Infectious hepatitis in association with pregnancy is described as rare[10,22,31,33,37,46]. However, Ingerslev and Teilum[20] have recently reported a series of 91 cases. With very little effort, this writer has learned of 12 unreported cases in addition to the 4 seen at this hospital[2,16,30,39,43]. These 16 cases and their end results are listed in Table 1. There are no large statistics on such incidence available, chiefly because the disease is not reportable by law in most states. Unfortunately, too, many physicians do not record their experiences, particularly with the milder forms of hepatitis.

TABLE 1.—TRIMESTER OF INFECTION AND END RESULT OF GESTATION IN 16 PREGNANT WOMEN WITH INFECTIOUS HEPATITIS

Source	*Patient*	*Trimester with Infection*	*Baby*
USNH, Chelsea	16582	second	normal term birth
	12027	third	stillborn; antepartum death due to erythroblastosis
	17209	third	normal term birth
	17547	second	stillborn; intrapartum death due to erythroblastosis
	19162*	first	normal term birth
Anonymous (1)	A	first	spontaneous abortion
	B	first	spontaneous abortion
	C	third	normal term birth
Barker (2)	A	second	normal term birth
	B	third	normal term birth
Hernquist (16)	A	third	stillborn, antepartum death (no autopsy reported)
McLaughlin (30)	A	second	normal term birth
	B	second	normal term birth
	C	second	normal term birth
Stouffer (39)	A	second	normal term birth
Wilson (43)	A	third	normal term birth

* Care for hepatitis in pregnancy elsewhere; patient now under our care for a second pregnancy.

In view of the great variability in severity of hepatitis in general, it is easy to appreciate the many problems

FIG. 1.—Liver biopsy showing subacute hepatitis in a pregnant patient (Case No. 16582) two weeks after liver function tests had returned to normal results.

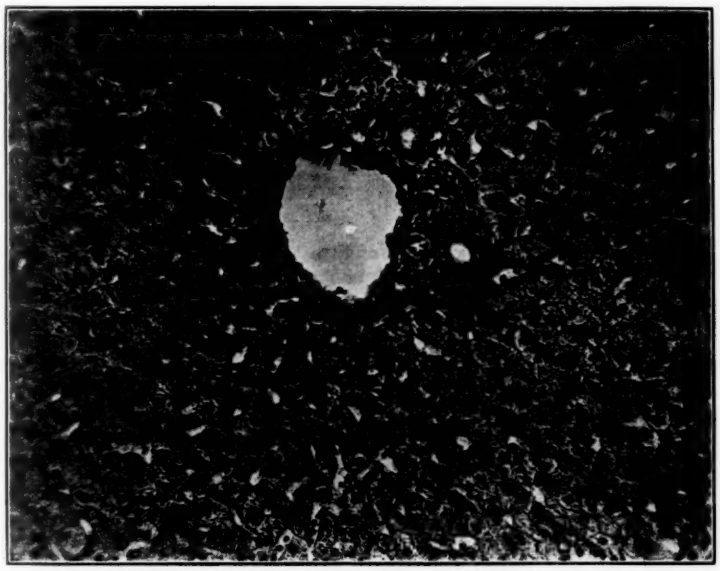

FIG. 2.—Liver biopsy showing subacute hepatitis from same patient as in Fig. 1, and at same time.

TABLE 2.–THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE IN PREGNANCY

Diseases Peculiar to Pregnancy

Eclampsia
Pre-eclampsia

Diseases Coincidental with Pregnancy

A: *Infectious*

Viral
Infectious Hepatitis
Homologous Serum Jaundice
Infectious Mononucleosis

Bacterial
Tuberculous hepatitis and cholangitis
Typhoid Fever
Typhus Fever
Pneumonia
Hepatic Abscess

Spirochetal
Syphilis; secondary, tertiary
Weil's Disease, other leptospiroses

Parasitic-Exotic
Echinococcus
Amebiasis
Yellow Fever
Malaria
Dengue
Ankylostomiasis
Round Worms

B: *Metabolic*
Starvation

C: *Chemical Intoxication*
Chlorinated hydrocarbons (Chloroform, carbon tetrachloride)
Phenyl hydrazine
Dinitrophenol
Trinitrotoluene
Phosphorus
Antimony
Arsenic
Gold
Cinchophen
Sulfonamides

D: *Other Toxins*
Bacterial Infections
Mushroom (Amanita phalloides)
Snake Venom

E: *Cirrhosis*

F: *Malignant Disease and Neoplasia*
Carcinoma of liver, bile duct, common duct, head of pancreas, and gall bladder
Secondary metastatic carcinoma of liver
Leukemia
Hodgkin's Disease
Lymphosarcoma

G: *Hematologic*
Transfusion reaction
Paroxysmal hemoglobinuria
Sickle Cell Anemia
Splenic Anemia

H: *Biliary Tract*
Cholecystitis
Cholelithiasis
Common duct stone
Pancreatitis

that hepatitis can pose in obstetrical practice. Javert and Morrison[22] discuss anicteric jaundice and mention Irving's[21] study of vomiting in pregnancy with an 80% incidence of elevated icteric index, but no jaundice. There are frequent epidemics of minor gastrointestinal illnesses at all seasons. Many pregnant women have gastrointestinal complaints that are dismissed as functional or psychogenic. When these facts are assessed against the common occurrence of hepatitis, particularly without symptoms or overt jaundice[7,32], there is reason to suspect that it occurs commonly in pregnancy as well.

PATHOLOGY. There are detailed reports of the pathology that define the anatomical chemical and physiological changes so completely that repetition is pointless[27,28,29,44]. Figures 1 and 2 are representative of typical changes in the liver during the recovery phase. The pathological diagnosis was subacute hepatitis. The visible incomplete resolution is significant in that the liver function tests had returned to normal 2 weeks previously.

SYMPTOMS. Lucké[27] and Wood[44] have described the clinical varieties and the clinical course in great detail, with especial reference to the pathological conditions present. Zondek and Bromberg[46] give perhaps the most attention to the clinical picture in pregnancy. However, hepatitis in pregnancy does not differ from hepatitis in the non-pregnant.

DIAGNOSIS. In the absence of a specific serological test, the diagnosis is established by exclusion. The new skin test promises to be of use but needs further substantiation[15]. Any condition resulting in jaundice and change in liver function is suspect as hepatitis. In Table 2 are listed the conditions which have or conceivably may occur coincidentally with pregnancy[3,6,8,23,40,44,45].

Eclampsia may result in liver changes. However, there is no typical lesion[12,42] and, in many instances, no liver changes have been noted on liver biopsy[19]. The studies of Ingerslev and Teilum[19] failed to reveal liver changes in pre-eclampsia. Only eclampsia appears to be a possible cause of jaundice among those conditions which are peculiar to pregnancy. Of interest is the recent case of Wilson[43] in which hepatitis and severe pre-eclampsia occurred coincidentally, the pre-eclampsia clearing after delivery to reveal the exact nature of the presenting syndrome.

Zondek and Bromberg[46] commented on the frequent simultaneous occurrence of Weil's disease and hepatitis in their epidemic. The routine use of the heterophile agglutinin test on the medical service at this hospital has frequently indicated the concurrence of infectious hepatitis and infectious mononucleosis.

PROGNOSIS. The only fatality in the 91 patients of Ingerslev and Teilum[20] was a patient who sought medical care only when she was moribund. In the presence of poor nutrition under lowered economic standards and subsequent interruptions of pregnancy, Zondek and Bromberg[46] lost 5 of their 29 patients. Except that spontaneous abortion[1] and premature[20,46] labor may occur as with any severe constitutional disease or prolonged febrile illness, hepatitis in pregnancy does not differ from hepatitis in the non-pregnant state. There are no specific maternal effects peculiar to pregnancy. Recovery is usually complete[11,28,35], although chronic cases are reported[7]. Lucké indicates evidence of a second primary infection in his experience[28]. Present evidence indicates no relationship between hepatitis and Laennec's cirrhosis, although both may occur simultaneously in the same patient.

Two case reports of fetal anomalies [24,25] raise the question of damage to the fetus when the infection occurs in the first trimester, as has been observed with rubella[14,41]. However, rubella does not always result in anomalies[9]. No similar correlation between hepatitis and anomalies has been found. Poliomyelitis does not increase the number of anomalies[13,42]. Smallpox vaccination during pregnancy does not increase the number of anomalies[36].

One patient reported by Ingerslev and Teilum[19], and another (19162) now under our care for her second pregnancy delivered normal term infants following hepatitis in the first trimester. Two babies with anomalies, reported by Ingerslev and Teilum[20], were noted after hepatitis in the second, not the first trimester. There is, therefore, no evidence to incriminate viral hepatitis as a cause of anomalies *per se*.

Except to deny any role of viral hepatitis in the production of anomalies, nothing else can be said of possible effects on the fetus. These babies appear normal at birth, but it is possible that they may have anicteric hepatitis or are carriers. In the study of an institutional epidemic reported by Capps and Stokes[7] among children under 3 years of age, the disease was generally without jaundice and persisted 6 to 8 months. There is need to study these babies and a control series by such means as the skin test, liver function studies and stool cultures for virus.

In severe viral hepatitis resulting in acute yellow atrophy of the liver, De-Lee and Greenhill report stillborn babies to have "liver changes"[10]. Javert and Morrison[22] mention one such stillborn infant whose liver showed fatty degeneration. Two of our babies were stillborn as a result of concurrent severe erythroblastosis associated with moderate hepatitis. No evidence of liver change unrelated to erythroblastosis was found.

TREATMENT. There is no indication to interrupt the pregnancy by abortion or induction of labor. Actually, these procedures are contraindicated, and the additional stress resulting from operation, trauma and anesthesia may be fatal.

Metabolic and electrolytic requirements have first priority. A search for possible chemotoxins is important; not only can they result in severe hepatitis *per se*[27,28,29,41], but they have a cumulative effect and a synergistic action[5] in the presence of infection. The importance of diet during and prior to the infection has been stressed[18]. A high protein and carbohydrate diet is essential, in addition to the liberal intake of B-complex vitamins.

When surgery becomes necessary, the use of adrenal cortical extract has been suggested by Mickal[31], although his rationale is not elaborated. Lucké found no morphological changes in the adrenal[27], but morphology is a poor indicator of functional levels. One of our patients (A.M.S., 12027) was delivered by cesarean hysterectomy for obstetric indications. She went into shock disproportionate to blood loss, this state persisting despite intensive therapy until adrenal cortical extract was given. This experience is not conclusive, but it is suggestive.

PROPHYLAXIS. Gamma globulin gives passive immunity[38]. Assuming a mechanism similar to that proposed by Herrman[17], it may also give a passive-active immunity. This suggests the possible use of gamma globulin as a prophylactic measure for pregnant women during a known epidemic. However, we must admit that there is no real reason to give preference to the pregnant over the non-pregnant woman in this respect.

It is interesting that Beck[4] believes his failure to encounter hepatitis in

his private practice was the result of his attention to and emphasis on diet during pregnancy. Certainly, the reported experience indicates a better prognosis for those patients with adequate diet prior to their infection.

Discussion. Infectious hepatitis is not an uncommon complication of pregnancy. Its true incidence is unknown and will remain unknown until reporting of this disease is legally required. Such action is recommended as a public health measure.

When hepatitis occurs in pregnancy, some cases will end by spontaneous abortion or premature labor. The maternal effects are not otherwise influenced by the presence of a pregnancy. Any interference with the pregnancy is contraindicated. The knowledge of possible fetal effects is incomplete, although it is important to state that there is no evidence to indict hepatitis as a direct cause of anomalies. Babies born of mothers with hepatitis need further study by investigative measures not generally available.

Attention to dietary, metabolic and electrolytic requirements is the most important therapeutic consideration. During periods of stress, as during necessary surgery, the use of adrenal cortical extract may be lifesaving. Its use deserves further trial for evaluation. Gamma globulin may be employed as a prophylactic measure.

Summary and Conclusions. 1) There are reported 4 cases of infectious hepatitis complicating pregnancy as observed by the author and 12 additional cases, 10 of them gathered from the experience of colleagues and 2 from published reports, a total of 16 cases.

2) There occurred 2 spontaneous abortions, 3 stillbirths and 11 normal term births.

3) A review of the literature discloses no evidence that hepatitis causes any anomalies or defects in the fetus.

4) The maternal mortality is apparently no greater than in the nonpregnant with hepatitis.

5) Infectious hepatitis complicating pregnancy is, therefore, no indication for therapeutic abortion or induction of labor.

REFERENCES

1. Anonymous: Discussion of Stouffer[39], and Hernquist and Eavey[16]: Proc. 1st Army-Navy-Air Force Obs-Gyn Seminar, Ft. Sam Houston, Texas, March 27, 28, 29, 1952.
2. **Barker, R. H.**: Personal communication.
3. **Beck, A. C.**: Obstetrical Practice, 5th ed., Baltimore, Williams & Wilkins, 1951.
4. Idem: Personal communication.
5. **Bennett, G. A., Drinker, C. K.,** and **Warren, M. F.**: J. Indust. Hyg. & Toxicol., **20,** 97, 1938.
6. **Boyd, W.**: A Textbook of Pathology, 3rd ed., Baltimore, Williams & Wilkins, 1938.
7. **Capps, R. B.,** and **Stokes, J., Jr.**: J. Am. Med. Assn., **149,** 557, 1952.
8. **Cecil, R. L.**: A Textbook of Medicine, 6th ed., Philadelphia, W. B. Saunders Co., 1943.
9. **Cordes, F. C.**: Arch. Ophth., **42,** 596, 1949.
10. **DeLee, J. B.,** and **Greenhill, J. P.**: Principles and Practice of Obstetrics, 9th ed., Philadelphia, W. B. Saunders Co., 1949.
11. **Dible, J. H., McMichael, J.,** and **Sherlock, S. P. V.**: Lancet, **2,** 403, 1943.
12. **Dieckmann, W. J.**: Toxemias of Pregnancy, 2nd ed., St. Louis, C. V. Mosby Co., 1952.
13. **Greenhill, J. P.**: 1951 Year Book of Obstetrics and Gynecology, Chicago Year Book Publishers, Inc., 1951.
14. **Gregg, N. M.**: Trans. Ophth. Soc. Australia, **3,** 35, 1942.
15. **Henle, G., Drake, M. E., Henle, W.,** and **Stokes, J., Jr.**: Proc. Soc. Exper. Biol. & Med., **57,** 603, 1950.
16. **Hernquist, W. C.,** and **Eavey, J. L.**: Proc. 1st Army-Navy-Air Force Obs-Gyn Seminar, Ft. Sam Houston, Texas, March 27-29, 1952.
17. **Herrman, C.**: Arch. Pediat., **39,** 607, 1922.
18. **Hinsworth, H. P.,** and **Glynn, L. E.**: Clin. Sci., **5,** 93, 1944.

19. **Ingerslev, M., and Teilum, G.:** Acta obst. gyn. Scand., **25,** 339, 1946.
20. Idem: Ibid., **31,** 74, 1951.
21. **Irving, F. C.:** Virginia Med. J., **36,** 537, 1940.
22. **Javert, C. T., and Morrison, R. C.:** Texas State Med. J., **47,** 137, 1951.
23. **Karsner, H. T.:** Human Pathology, 6th ed., Philadelphia, J. B. Lippincott Co., 1942.
24. **Kass, A.:** Acta Paediatrica, Stockholm, **40,** 239, 1951.
25. **Kellogg, C. S.:** and **Wesp, J. E.:** Am. J. Obst, & Gynec., **62,** 1153, 1951.
26. **Lesse, M. F., and Hamolosky, M. W.:** J. Am. Med. Assn., **147,** 727, 1951.
27. **Lucké, B.:** Am. J. Path., **20,** 471, 1944.
28. Idem: Ibid., **20,** 595, 1944.
29. **Lucké, B., and Mallory, T.:** Ibid., **22,** 867, 1946.
30. **McLaughlin, J. L.:** Personal communication.
31. **Mickal, A.:** Am. J. Obst. & Gynec., **62,** 409, 1951.
32. **Neefe, J. R.:** Ann. Int. Med., **31,** 857, 1949.
33. **O'Connel, W. T.:** Am. J. Obst. & Gynec., **63,** 449, 1952.
34. **Opie, E. L.:** J. Exper. Med., **12,** 367, 1910.
35. **Roholm, K., and Iverson, P.:** Acta path. et microbiol. Scandinav., **16,** 427, 1939.
36. **Snyder, S. S.:** Am. J. Ophth., **34,** 1713, 1951.
37. **Stander, H. J.:** Williams' Obstetrics, 8th ed., New York, Appleton Century, 1941.
38. **Stokes, J., Jr., Drake, M. E., Capps, R. B., Ward, C. S., Mills, O., and Kitts, A. W.:** J. Am. Med. Assn., **147,** 714, 1951.
39. **Stouffer, J. G.:** Proc. 1st Army-Navy-Air Force Obs-Gyn Seminar, Ft. Sam Houston, Texas, March 27, 28, 29, 1952.
40. **Strong,** R. P.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, 6th ed., Philadelphia, Blakiston Co., 1942.
41. **Swan, C., Tostevin, A. L., Moore, B., Mayo, H., and Black, G. H. B.:** Med. J. Australia, **2,** 201, 1943.
42. **Tenney, B., Jr.:** Personal communication.
43. **Wilson, K. M.:** Personal communication.
44. **Wood, D. A.:** Arch. Path. **41,** 345, 1949.
45. **Yater, W. M.:** Symptom Diagnosis, Regional and General, 4th ed., New York, Appleton Century, 1942.
46. **Zondek, B., and Bromberg, Y. M.:** J. Mt. Sinai Hosp., **14,** 222, 1947.

CORTISONE* AS AN ADJUNCT IN THE REHABILITATION OF THE HEMIPLEGIC PATIENT**†

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As a continuing study in methods of management of the patient with hemiplegia resulting from a cerebrovascular accident, it seemed possible that certain known effects of cortisone (psychomotor stimulation, increased sense of well being, obtundation of pain and depression of fibroblastic activity) might be utilized to influence the time-course of rehabilitation of such patients. Many hemiplegics complain of pain on passive motion of the involved extremities; in addition, they frequently exhibit mental depression of varying degree and, lacking motivation, do not enter enthusiastically into the retraining program. To overcome muscle spasm and pain, methods employing procaine injection, ethyl chloride spray, neostigmine, heat and hydrotherapy are commonly employed. In addition, a certain amount of psychotherapy for concomitant mental depression is also utilized. Since the multiple actions of cortisone might render such procedures unnecessary, it was thought of interest to explore the effect of orally administered cortisone as an adjunct to routine rehabilitation techniques[2,5].

Methods. Case selection in this study was considered from two aspects: (1) the residual cerebral and cardiovascular status, and (2) the possible risk of untoward effects from the administration of cortisone. Patients exhibiting severe encephalomalacia, ma-

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lignant arteriolonephrosclerosis, intractable coronary insufficiency or congestive heart failure, did not qualify as suitable subjects for this investigation. A history of diabetes mellitus or overt psychotic episode, the presence of active or chronic pulmonary tuberculosis, endocrine dyscrasia or severe renal disease were each considered to represent a contraindication to the use of cortisone. Hypertensive cardiovascular disease *per se* was not regarded as a contraindication, nor was a history of antecedent cerebrovascular or myocardial infarctive episodes. Most of the patients had hypertension or arteriosclerotic cardiovascular disease or both and the etiological basis for the cerebrovascular episode was either thrombosis or embolism. Other details of case selection were similar to those as noted in previous publications[1,4].

After initial evaluation, all patients were placed on the rehabilitation program as previously outlined[2,5]. In one group of patients, the administration of the drugs was by means of the "double blind" system where neither the physicians nor the patient had knowledge of the specific medication being dispensed (Series I). In this series, 3 groups of patients were studied; namely a group treated with: (1) cortisone, and (2) control group, the latter consisting of (a) a group given placebo, and (b) another given aspirin (for analgesic effect). In another group of cases, placebo medication preceded the administration of cortisone, so that the individual patients served as their own controls (Series II).

DESCRIPTION OF PATIENTS. *Series I.* Eight patients, selected by chance for inclusion in the cortisone treated group, had a mean age of 48.5 years (range 26 to 66 years) with a mean elapsed interval of 11.8 months (range 3 to 49 months) after the cerebrovascular accident before treatment was started. This group had been on the rehabilitation program for a mean of 35.5 days (range 3 to 106 days) before starting treatment. In the control group there were 10 patients, 5 each on placebo and aspirin. One patient in the placebo group died on the third day of observation, having sustained what appeared to be a sudden myocardial infarction with pulmonary edema while asleep. The mean age for the control group was 57.5 years (range 45 to 72 years) with a mean elasped interval of 9.5 months (range 5 to 27 months) after the cerebrovascular accident before treatment was started. This group had been on the rehabilitation program 33.7 days (range 5 to 107 days) before treatment was started. The characteristics of the patients are indicated in Table 1.

Series II. In addition, another group of 18 patients, mean age 58.2 years (range 47 to 64 years), was treated with cortisone. These served as their own controls by virtue of being placed on placebo therapy for variable periods of between 2 to 3 weeks before starting cortisone. The mean time post cerebrovascular accident for this group was 8.7 months (range 3.5 to 17.3 months).

TABLE 1.—CHARACTERISTICS OF PATIENTS STUDIED ON CORTISONE, ASPIRIN OR PLACEBO, AND REHABILITATION PROGRAM (SERIES I)

Patient Groups	*No.*	*Age Yrs.*		*Time from Cv Episode to Start R* Mos.*		*Time from Adm. to Rehab. to Start R* Days*	
		Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
Cortisone	8	48.5	26–66	11.8	3–49	35.5	3–106
Control	9	57.5	45–72	9.5	0.5–27	33.7	5–107
Aspirin	5	57.6	50–65	10.9	0.5–27	42.4	5–107
Placebo	4	57.3	45–72	8.0	0.5–17	25.0	7–48

ADMINISTRATION OF MEDICATION. In the "double blind" method, the medications were prepared so that the cortisone, placebo and aspirin tablets resembled each other in size, shape, taste and color. Actual administration (oral) of the preparations was overseen by the physician, who checked the work sheet on each patient daily. Patients were started on a dose of 200 mg. of cortisone for the first 2 days with progressive diminution to a maintenance dose of 50 mg. daily through the third week. Placebo was substituted for the fourth week and observations were continued from the fifth through the eighth week after commencement of the administration of medication. The dosage details are noted in Table 2.

In addition, all patients were placed on a low salt diet with 3 gm. of potassium chloride orally a day in divided doses. After control pre-medication studies, serial observations

were also made of the electrocardiogram, hemogram, as well as urines for glucose and formed elements. Daily body weights, as well as blood pressures for at least 3 determinations (supine position in the morning before getting out of bed), were also recorded. Serial serum sodium and potassium levels were not followed.

METHOD FOR MEASURING AND RECORDING FUNCTIONAL TESTS. Serial analysis of activities of daily living, passive range of motion and muscle strength were performed before, at weekly intervals during, at the end of drug administration, as well as one month after termination, and percentage gain was calculated as previously described[1, 4].

It must be realized, however, that while such methodology attempts to be objective, it is recognized that it does not quantitate (a) the inclination of a patient to perform such activities, and (b) ability to perform them in terms of frequency.

TABLE 2.—SCHEDULE OF ORAL CORTISONE DOSAGE DURING COURSE OF STUDY

		Daily Total (mg.)	*Schedule*
1st Week	2 days	200	50–50–50–50
	5 days	150	50–50–50
2nd Week		100	25–50–25
3rd Week		50	12.5–25–12.5
4th Week		Placebo	1 tablet 3x daily
5th to 8th Weeks		No medication while still under observation on training program	

TABLE 3.—ACTIVITIES OF DAILY LIVING: SUMMARY OF RESULTS (SERIES I)

Patient Groups	*Before R, % of Normal*		*One Month After R, Discontinued % of Normal*		*% of Maximum Possible Gain Achieved*	
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
Cortisone	52.7	26.9–82.9	74.7	46.1–95.0	48.9	15.6–84.2
Control	36.6	16.9–80.7	63.5	41.5–92.0	43.9	21.0–82.4
Aspirin	43.2	16.9–80.7	70.3	41.5–92.0	48.2	21.0–82.4
Placebo	28.4	18.7–38.2	55.1	44.0–81.5	38.6	26.5–70.0

Results. EFFECT ON FUNCTIONAL TESTS. In general, there were no striking differences noted in any of the functional test analyses among the cortisone and control groups in Series I, and, further, there were no significant differences noted at the end of the fourth week compared with those found at the end of the eighth week.

In activities of daily living, it can be noted (Table 3), that the percentage of maximum possible gain achieved for the cortisone group (Series I), was 48.9%, while that for the control group was 43.9% (aspirin group 48.2%; placebo group 38.6%).

In terms of muscle strength, the apparent better response of the control group over the cortisone treated group (Series I), especially for the arm-forearm and hand, is explained by the fact that the starting level of muscle strength was low in the cortisone treated group, (19.3% of normal for arm-forearm compared to 42.7% in the control group), and 17.7% of normal for the hand as compared with 30.9% for the control group. This bears out the well delineated clinical impression that hand function returns very poorly, even in an excellent rehabilitation program. There were no significant differences in per cent of maximum possible gain in the thigh-leg (22.1% com-

pared to 20.6%) and the foot (15.7% compared to 16.1%) (Table 4).

In those individuals where muscle spasm was a factor, there was no clinical effect noted as a result of any of the medications. Electromyograms, however, were not serially performed.

Analysis of passive range of motion (Series I), indicates that there were no losses in per cent of normal, nor were there any significant differences between the cortisone and control groups. There was a gain of 8.3% for the cortisone treated group compared with a gain of 7.1% for the control group in the arm-forearm (Table 5).

However, since such objective clinical tests do not afford a direct measure of the psychomotor, sensory or emotional status of the patient, such methods may need supplementation for drug evaluation in this syndrome.

In the self-controlled group of 18 patients (Series II) treated with cortisone, there was noticeable alleviation of pain, and diminution of mental depression and anxiety. The increase in co-operativeness in 6 of these, while on cortisone, (mean time 4.3 months after the cerebrovascular episode), resulted in a more enthusiastic entrance into the training program. The patients who responded most strikingly ranged from 3 to 5 months post-cerebrovascular episode, and had apparently ceased making progress on the rehabilitation program when they were placed on the study. In these it

TABLE 4.–MUSCLE STRENGTH: SUMMARY OF RESULTS (SERIES I)

		Arm—Forearm		*Hand*		*Thigh—Leg*		*Foot*	
Patient Groups	*No.*	*Bef.* R̄ % *Nor**	*Fin. Imp.* %†	*Bef.* R̄ % *Nor*	*Fin. Imp.* %	*Bef.* R̄ % *Nor*	*Fin. Imp.* %	*Bef.* R̄ % *Nor*	*Fin. Imp.* %
Cortisone	8	19.3	17.9	17.7	5.8	40.9	22.1	17.2	15.7
Control	9	42.7	25.8	30.9	34.1	49.5	20.6	23.2	16.1
Aspirin	5	44.0	34.5	27.2	39.3	48.3	20.5	15.9	20.3
Placebo	4	41.1	14.9	35.6	27.7	51.0	20.7	32.3	10.8

* Before treatment in percentage of normal muscle strength for that part
† Final improvement in percentage of maximum possible gain achieved one month after drug therapy discontinued.

TABLE 5.–PASSIVE RANGE OF MOTION: SUMMARY OF RESULTS (SERIES I)

		Mean Percentages of Normal Passive Range of Motion								
		Arm—Forearm			*Hand*			*Thigh—Leg*		
Patient Groups	*No.*	*Bef.** R̄	*Aft.*† R̄	*Gain or Loss*	*Bef.* R̄	*Aft.* R̄	*Gain or Loss*	*Bef.* R̄	*Aft.* R̄	*Gain or Loss*
Cortisone	8	75.1	83.4	8.3	98.6	98.6	0.0	81.2	91.8	10.6
Control	9	79.2	86.3	7.1	88.9	89.9	1.0	87.2	92.5	5.3
Aspirin	5	82.6	88.3	5.7	85.2	86.5	1.3	85.3	93.2	7.9
Placebo	4	74.9	83.8	8.9	93.5	94.0	0.5	89.7	91.7	2.0

* Before treatment
† After treatment: One month after drug therapy discontinued.

seemed that a distinct increment in functional capacity occurred during the course of cortisone therapy, although quantitative analyses of the tests, as described for Series I, were not performed.

EFFECT ON ELECTROCARDIOGRAM, BLOOD PRESSURE, URINARY GLUCOSE, AND BODY WEIGHT. In no instance, in either series, was there any progressive effect on the electrocardiogram in those patients who had electrocardiographic evidence of past myocardial infarction. This bears out previous studies[6], in which it was shown that cortisone caused no significant effect on the electrocardiogram with reference to standardized exercise tolerance in patients with coronary artery disease. Likewise, there were no significant rises in blood pressure in the cortisone treated group, although several of these had original significantly elevated systolic and diastolic levels. In only 2 individuals was there an intermittent slight glycosuria which appeared during the course of cortisone administration. In the post-medication period, these reverted to normal. There were no significant changes in body weight, nor were there any abnormal effects noted on the hemogram.

SUBJECTIVE EFFECTS. The most striking effect in the cortisone treated groups (in both Series I and II) seemed to be an apparent increased feeling of well-being and enhanced motivation. In some patients this bordered on euphoria. As a result, these entered into the training program with greater co-operation. In addition, there was significant diminution of pain in those instances where this was a limiting factor, especially in range of motion. This effect was more noticeable in the cortisone than in the aspirin treated group. It was not considered feasible to attempt quantitation of these subjective effects.

Discussion. A serious problem in rehabilitating the average hemiplegic is the one of motivation, since frequently mental depression is a prominent finding. Such individuals resist the retraining process, in part due to the fact that motion of the involved extremities causes great pain. By alleviating the mental depression, minimizing the pain in affected extremities and allaying anxiety, a consistent response was seen in patients receiving cortisone in that there was greater interest and fuller participation in the program. The number of patients studied is relatively small, but the clinically subjective effects were striking in those who were receiving cortisone.

From objective clinical analysis of activities of daily living, muscle strength, and range of motion, cortisone in addition to a training program did not appear to affect significantly the time-course of rehabilitation of the routine hemiplegic subject under the conditions outlined. However, the relatively small number of cases, the difficulty in obtaining comparable controls and the obvious acceleration of clinical functional response to rehabilitation in some patients under cortisone, would not permit final conclusions from these quantitative results. Moreover, the relief of pain and the striking changes in the psyche of individual patients afford proof of the value of such adjuvant therapy in selected cases.

No attempt was made to study intrinsic somatic muscle metabolism or joint lesions. Of considerable significance was the fact that none of the patients on cortisone sustained another cerebrovascular or myocardial accident. This does not confirm the findings of Cosgriff[3], who indicated that cortisone increased the thrombotic propensities of older patients. However, further studies, adequately controlled, are needed here. In addition, there

was no significant effect on blood pressure in those with elevated systolic and diastolic levels. The one death in the entire series occurred in a 64-year old male hemiplegic who had had a cerebrovascular accident 7 months previous to admission and whose electrocardiogram, in addition, showed changes compatible with a posterior wall myocardial infarction. This patient was receiving placebo and death occurred while he was asleep.

The effects of a program of rehabilitation are again demonstrated, and as has been our past experience, the greatest gains in all functional tests are demonstrated by those patients who present themselves for rehabilitation earlier in the post-cerebrovascular accident phase and before contractures, deformities and improper patterns of ambulation supervene.

It would appear that cortisone is of advantage in this connection by virtue of its known side actions; namely, increased psychomotor stimulation, sense of well-being, and analgesic effect. No significant rebound phenomena were observed following the discontinuance of such therapy.

Summary. Cortisone was administered orally in conjunction with a program of training in 26 hemiplegic patients. Eight patients received the drug according to the "double blind" system, (Series I) and 18 served as their own controls after having received a preliminary course of placebo therapy (Series II). The following observations and conclusions appear warranted:

1) In eight patients (Series I), no significant effect was noted on the time-course of rehabilitation compared with controls (those receiving aspirin and placebo) as revealed by serial quantitative analysis of activities of daily living, range of motion and muscle strength. The small number of cases and the difficulty in obtaining comparable controls do not permit final conclusions from this analysis. In another group of 18 patients (Series II), an acceleration in the rate of improvement in functional capacity was clinically observed in 6 cases during cortisone administration.

2) Because of its striking effect in promoting a sense of well-being, improving motivation and diminishing pain, cortisone is a useful adjunct for enhancing participation in a program of rehabilitation in properly selected cases.

3) Under the conditions of dosage, patient selection, management and observation of this study, cortisone had no significant effect on electrocardiogram, hemogram or blood pressure, and did not appear to induce thrombo-embolic phenomena.

In addition, we wish to thank Miss Vilma Smith, B.Sc., for technical assistance, and Miss Kate Elend, R.P.T., Miss Marguerite Fortman, R.P.T., Miss Ronnie Silverman, R.O.T., and Miss Kay Hinckley, R.P.T., for performing the functional tests.

REFERENCES

1. **Benton, J. G., Brown, H.,** and **Rinzler, S. H.:** Am. Heart J., **42**, 719, 1951.
2. **Benton, J. G.,** and **Rusk, H. A.:** Modern Concepts of Cardiovascular Disease, **19**, 12, p. 85, 1950.
3. **Cosgriff, S. W.:** J. Am. Med. Assn., **147**, 924, 1951.
4. **Rinzler, S. H., Brown, H.,** and **Benton, J. G.:** Am. Heart J., **42**, 710, 1951.
5. **Rusk, H. A., Deaver, G. G., Covalt, D. A., Marks, M., Benton, J. G.** and **Turnblom, M.:** Hemiplegia and Rehabilitation; Seminar, Jan.-Feb., 1952 (Sharp and Dohme), p. 1.
6. **Russek, H. I.,** and **Zohman, B. L.:** Effect of Cortisone on Electrocardiographic Response to Exercise in Patients with Coronary Disease. (To be published).

THE EFFECT OF BUTAZOLIDIN (PHENYLBUTAZONE) ON WATER AND ELECTROLYTE EXCRETION*

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Butazolidin or 3, 5-dioxo-1, 2-diphenyl-4-n-butylpyrazolidin (phenylbutazone) has received widespread attention in the European literature since 1949[1,3,4,8,11], and more recently in the American literature[5,6,7,9], as a potent and effective analgesic and antipyretic agent in the treatment of rheumatic and allied disorders.

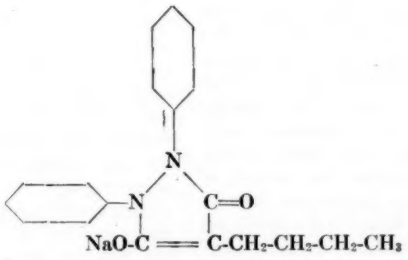

Fig. 1.—Butazolidin.

The drug was first synthesized in 1948 as a solubilizing agent for aminopyrine to facilitate parenteral administration of this relatively water insoluble drug[11]. Shortly thereafter a parenteral preparation containing 15% aminopyrine and 15% phenylbutazone as the solubilizing agent was released in Europe for investigation under the trade name of Irgapyrin. Following a series of favorable clinical reports concerning the value of Irgapyrin as a potent analgesic agent in the rheumatic disorders[1,3,4,8], an identical preparation in oral and parenteral form was issued for investigation in the United States under the trade name of Butapyrin. While these preparations were favorably accepted, the toxic properties of aminopyrine were still evident. Since the effectiveness of phenylbutazone alone as an analgesic agent had been noted early, experimental trials with this drug were made and the drug has been released.

Several investigators have alluded to the sodium and water retaining effect of Irgapyrin and Butapyrin and the occasional development of edema[2,-

* The Butazolidin was furnished through the courtesy of the Geigy Company, New York, New York.

[5,6,9]. This aspect, however, has been largely minimized or neglected with the summary statement that these drugs are contraindicated in patients who are prone to edema formation. There are no published reports yet available which deal with the action of phenylbutazone on water and electrolyte excretion. This problem was dramatically brought to our attention when one of our patients (Case No. 1) receiving Butazolidin for rheumatoid arthritis was admitted with acute pulmonary edema to which she almost succumbed.

The more extensive clinical trials of Butazolidin by our group will be reported separately by Warner *et al.*[10]. It is the purpose of this paper to describe 4 cases in which studies of the water and electrolyte excretion were made in hospitalized patients receiving Butazolidin.

Case Reports. Case 1. B. C. was a 55-year old white female with chronic rheumatoid arthritis of approximately 20 years' duration. Her health was otherwise good and there had been no prior evidence of cardiovascular or renal disease. Five days prior to admission she was given Butazolidin, 0.2 gm. t.i.d. without regard to salt intake. She awoke suddenly the morning of admission with marked dyspnea, cough and pink, frothy sputum. Examination revealed the patient to be *in extremis* with severe pulmonary edema. Over the succeeding 12 hours she slowly responded to rapid digitalization, oxygen, morphine, tourniquets, orthopneic position, and salt restriction. Serial electrocardiograms were entirely within normal limits and fluoroscopy revealed the heart size at the upper limits of normal without specific chamber enlargement. There was neither clinical nor laboratory evidence of a myocardial infarction. She was found subsequently to have a wide pulse pressure (BP 120/60) and a faint but characteristic decrescendo murmur of aortic insufficiency. Within 7 days her weight dropped from 118 to 109 lbs. and there was a corresponding diuresis. Butazolidin therapy was subsequently reinstituted under hospital observation at the same dosage, but with a salt-poor diet containing about 2 gm. NaCl per day. During the first week of Butazolidin therapy, her urinary sodium excretion was less than 5 mEq./24 hrs. and increased to less than 25 mEq. on this regimen. Within 6 days she had gained 6 pounds, and then her weight remained stable. The addition of approximately 5 gm. NaCl per day to her diet caused a rapid increase of 6 more pounds with evidence of left ventricular failure. With the gain in weight, it is to be noted that the volume of packed red cells dropped from 41% to 34% and that the plasma volume (Evans Blue) rose from 2.09 to 2.89 liters. By this time, the venous pressure had risen sharply to 160 mm. water and the patient was in such marked left ventricular failure that the Butazolidin therapy was discontinued. With the discontinuance of Butazolidin, the institution of a salt-poor diet and the administration of one injection of a mercurial diuretic, there was a return to the baseline weight, volume of packed red cells, vital capacity and venous pressure within 7 days. Concomitantly there was a marked sodium, chloride and water diuresis. Repeated examinations of the urine failed to reveal any abnormality (Fig. 2).

Case 2. W. W. B. was a 52-year old white male with chronic rheumatoid arthritis of approximately 4 years' duration. He was treated with Butazolidin, 0.2 gm. q.i.d., with moderate relief of symptoms. While receiving this medication he gained 4 pounds. Studies of his renal and cardiac function revealed no abnormality. He was then transferred to the metabolic ward where the observations charted in Fig. 3 were made. For the first 7 days he was given placebos instead of Butazolidin, and it is to be noted that he steadily lost weight during this period. This was accompanied by a sodium and chloride diuresis. This represents a diuresis following the cessation of the Butazolidin therapy. On the 8th day the medication was resumed and there was a steady weight increase until a plateau was reached on the 13th day. The weight gain was accompanied by decreased urine volume and sodium and chloride excretion. The urinary creatinine output did not change nor was the glomerular filtration calculated from the endogenous creatinine clearance altered. On the 16th day the patient was given 1 cc. Mercuhydrin intramuscularly. There was a sharp rise in the urine volume and in the excretion of sodium and chloride. There was also a loss of about 3 pounds in body weight which was regained after several days. On the 20th day the Butazolidin was discontinued. Loss of weight and diuresis similar to that observed at the beginning of the experiment took place.

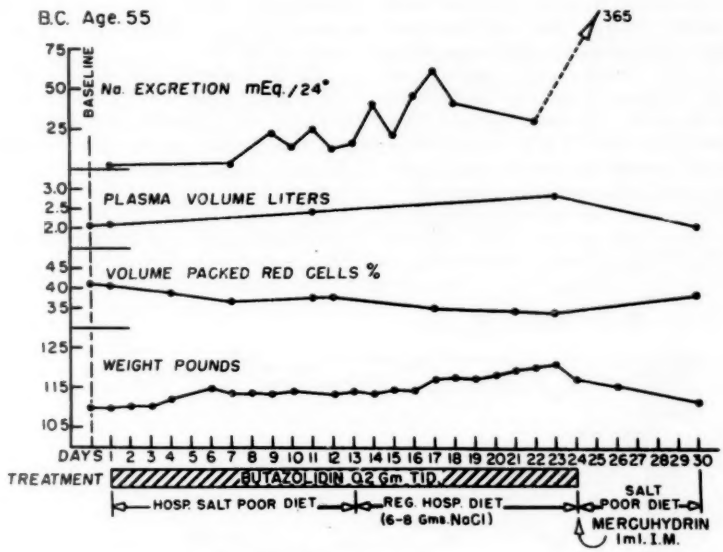

FIG. 2.—Sodium excretion, plasma volume, volume packed red cells and weight changes in Case 1.

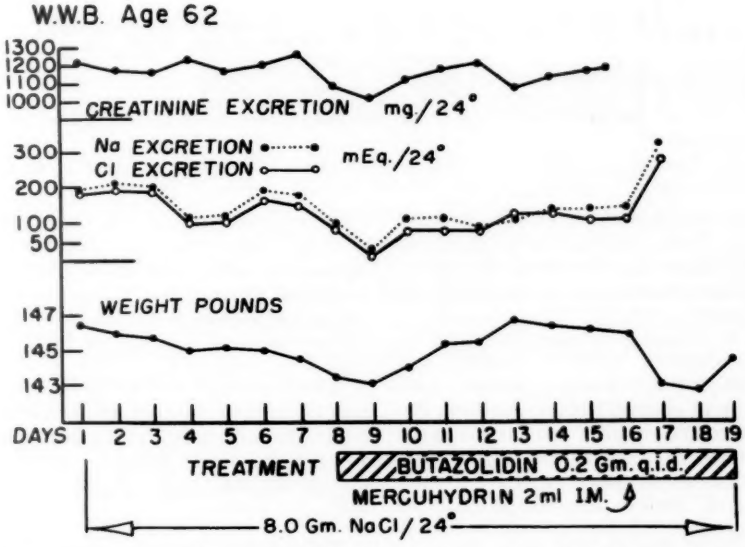

FIG. 3.—Creatinine, sodium and chloride excretion and weight changes in Case 2.

CASE 3. W. P. was a 28-year old white male who was convalescing from an attack of acute rheumatic fever. At the time of the study he was quite asymptomatic and was being observed on the ward while ambulatory. There was no evidence of cardiac insufficiency or renal involvement. He was eating a hospital salt-poor diet plus 5 gm. NaCl daily. After a control period he was given 0.2 gm. Butazolidin q.i.d. His weight increased 3 pounds and there was an accompanying drop in urine volume and sodium and chloride excretion (Fig. 4). Again there was no change edema developed. Fortunately she stopped taking the drug and diuresis followed mercurial therapy. She was later admitted for study (Fig. 5).

While taking Butazolidin, 0.2 gm. q.i.d., with a salt-poor diet of about 2 gm. NaCl per day, she gained 3 pounds in 8 days. During this period her urinary sodium excretion dropped from about 100 mEq./24 hrs. to less than 10 mEq./24 hrs. When 5 gms. of NaCl were added to her daily intake she gained an additional 12 pounds over the next 15 days. Concomitantly her vital capacity

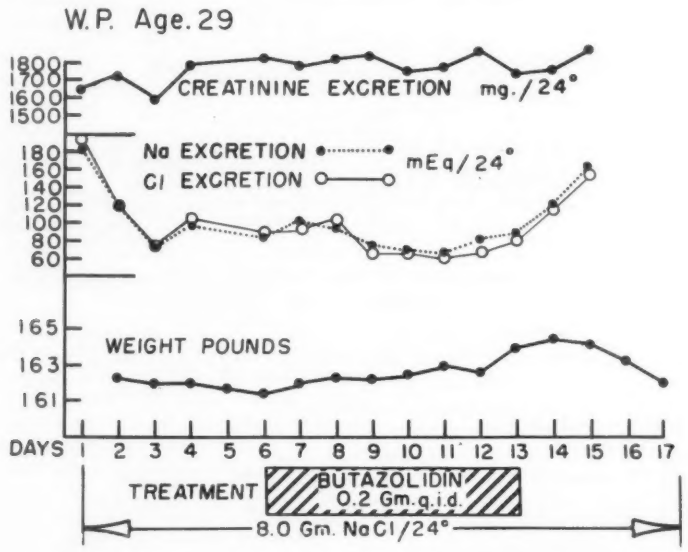

FIG. 4.—Creatinine, sodium and chloride excretion and weight changes in Case 3.

in the serum creatinine level or urinary creatinine output. Upon cessation of the Butazolidin therapy there was the usual diuresis of NaCl and water and a loss of weight to the base line level.

CASE 4. F. S. was a 56-year old white female with diabetes mellitus, obesity, and hypertensive cardiovascular disease with cardiomegaly of about 15-years' duration. Because of exertional dyspnea and diurnal pedal edema she had been digitalized 2 years previously with relief of symptoms. For 10 years she had had typical rheumatoid arthritis which had responded inadequately to salicylates. Because of this she was given Butazolidin, 0.2 gm. q.i.d., with instructions to follow a salt-poor diet. At the time Butazolidin was instituted, there was no evidence of cardiac decompensation or of renal insufficiency. In 4 days she gained 17 pounds and peripheral dropped from 2.3 to 1.4 liters; the venous pressure rose from 140 mm. to 225 mm. water; the calcium gluconate circulation time increased from 22 to 32 seconds; the plasma volume rose from 3.70 to 4.20 liters; and the volume of packed red cells fell from 53 to 44%.

Upon cessation of Butazolidin therapy there was a marked diuresis of sodium, chloride and water with a return to the baseline values for weight, vital capacity, venous pressure, circulation time and volume of packed red cells.

Discussion. After a rather extensive clinical experience with Butazolidin as an antirheumatic agent[10], it is anticipated that this drug will be used extensively. Consequently it is important

that the potential hazards of the drug be recognized. In our experience, weight gain has been a common complication. We have seen 2 instances of ulcerative stomatitis and only rarely have nausea and vomiting been precipitated by the drug. In the majority of patients receiving Butazolidin, fluid retention is not a serious complication. They may gain up to a few pounds, but their weight will then remain constant until the drug is discontinued or the salt intake is reduced. It should be emphasized that, in patients subject to fluid retention, Butazolidin therapy is hazardous if the proper precautions of a low salt diet and mercurial diuretics are not employed.

From the data presented it is apparent that the weight gain results from sodium chloride and water retention. In the 4 cases studied in detail, as well as in others which are not presented in this paper, Butazolidin therapy was accompanied by a sharp drop in sodium chloride and water excretion. The glomerular filtration as measured by the endogenous creatinine clearance does not change. This suggests that the decreased excretion is due to increased tubular reabsorption. The effect on the tubule is probably not mediated by the adrenal cortex since phenylbutazone therapy caused no drop in the eosinophile count[7].

Potassium excretion is unaffected by this agent.

Contrary to the experience of Kuzell *et al.*[7], the edema in many of our patients responded to a mercurial diuretic while the patients were receiving Butazolidin. This is illustrated in our second patient (Fig. 3).

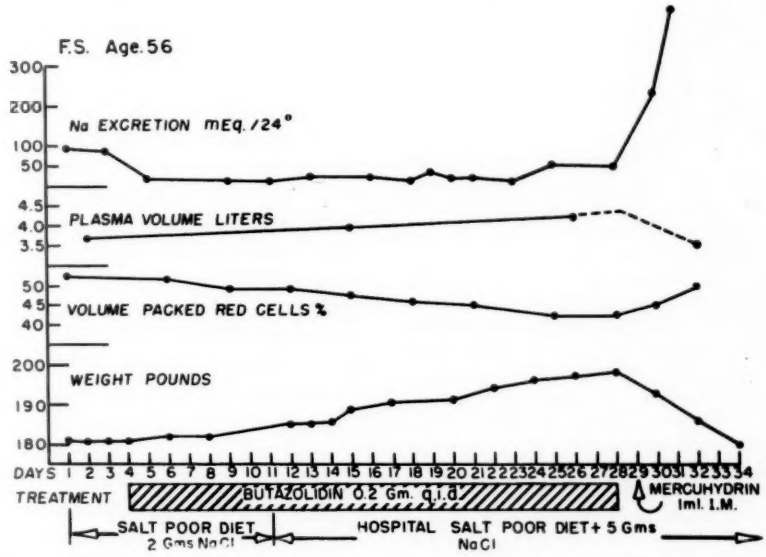

FIG. 5.—Sodium excretion, plasma volume, volume packed red cells and weight changes in Case 4.

Summary. 1. In 4 hospitalized patients, Butazolidin therapy caused decreased water and NaCl excretion, and, in 2, severe congestive failure was precipitated.

2. The glomerular filtration and potassium excretion were unchanged.

3. This effect of Butazolidin is probably mediated through a direct renal tubular mechanism.

REFERENCES

1. **Belart, W.:** Schewiz. med. Wchnschr., **79,** 582, 1949.
2. **Fabre, J.,** and **Mach, R. S.:** Schweiz. med. Wchnschr., **81,** 473, 1951.
3. **Fischer, H.:** Neue med. Welt, **1,** 1063, 1950.
4. **Kienle, H.:** München. med. Wchnschr., **92,** 885, 1950.
5. **Kuzell, W. C.,** and **Schaffarzick, R. W.:** Stanford Med. J., **9,** 194, 1951.
6. Iidem: Preliminary Report on Butapyrin prepared for Geigy Pharmaceutical Co., 1951.
7. **Kuzell, W. C., Schaffarzick, R. W., Brown B.,** and **Mankle, E. A.:** J. Am. Med. Assn., **149,** 729, 1952.
8. **Loewenhardt, F.:** Deutsche med. Wchnschr., **75,** 459, 1950.
9. **Steinbrocker, O.:** Preliminary Report on Butapyrin prepared for Geigy Pharmaceutical Co., 1951.
10. **Warner, R. S., Nunemaker, J. C.,** and **Jager, B. V.:** To be published.
11. **Wilhelmi, G.:** Schweiz. med. Wchnschr., **79,** 577, 1949.

THE TREATMENT OF ACUTE HYPOTENSIVE STATES WITH l-NOREPINEPHRINE*

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In recent years the experimental physiologist and the clinician alike have centered great interest in the phenomenon of "irreversible shock"[45]. Clinically, the condition may be found after very massive injury; more frequently, it comes on after an initial period of favorable response to therapy, when the patient suffers some additional strain such as operation, infection, myocardial infarction, or pulmonary embolism. Massive replacement with blood has usually failed[35,45]. Initially there is an over all increase in vascular tonus which becomes progressively impaired until, in the last, or "irreversible" phase, there is a loss of small vessel tone and pooling of blood in these channels[26]. Under these circumstances the administration of l-norepinephrine (noradrenaline, Arterenol, or Levophed**) may be a rational therapy for shock.

It is the purpose of this publication to report the results of administering norepinephrine to a group of patients in acute peripheral circulatory collapse from various causes with particular emphasis on the treatment of those cases complicating myocardial infarction and pulmonary embolism.

Methods. Solutions of l-norepinephrine were prepared by adding 4.0 mg. of 1:1000 norepinephrine to 1000 cc. 5% glucose in water. The pressor effect produced by intravenous drip infusion determined the rate of administration. In a few cases when a failure to obtain a satisfactory elevation of blood pressure occurred the concentration of the remaining solution was increased by adding an additional 4.0 to 8.0 mg. of 1:1000 norepineophrine. No attempt was made to determine the exact amount of the drug given per unit of time since the study was being carried out entirely at the clinical level. In all cases the object was to maintain blood pressures at optimum levels. Treatment was started immediately after detection of the shock state in the majority of cases. Some inadvertent delay occurred in a few instances.

A total of 22 patients were treated; of these 5 had suffered acute pulmonary emboli; 6 had acute myocardial infarctions; 1 had been given intravenous aminophyllin in the treatment of congestive heart failure; 2 cases complicated the use of Hexamethonium; 1 individual had an incompatible blood transfusion reaction; 2 persons with severe coronary insufficiency had post-operative shock; and one patient with refractory paroxysmal auricular tachycardia was complicated by shock. Profound shock was encountered in a case of acute leukemia with massive hemorrhage, in another individual with extensive second and third degree burns, and in chronic cor pulmonale with acute barbiturate intoxication. This latter patient was further complicated by marked cardiac decompensation.

All the cases were followed by frequent blood pressure and cardiac rate determinations at 5- to 15-minute intervals after a relative degree of stabilization had been reached. Serial electrocardiographic tracings, when possible, were obtained. In some, it was not possible to obtain them after therapy was

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** The Levophed was supplied by the Winthrop-Stearns Company.

TABLE 1.—RESULTS OF TREATMENT

Name	*Age*	*Sex*	*BP at Onset*	*Response to Norepinephrine at Onset*	*Amt. of Drug given in mg.*	*Length of time in Hours*	*Side Reactions to Drug*	*Etiology of Shock*	*Summary and End Result*
W. P.	71	M	0/0	130/80	44	72	None	Incompatible Blood transfusion	Satisfactory result. Norepinephrine life saving. In spite of transient oliguria for 4 days Pt. developed no ill effect from fluid administration. NPN rose to 150 mg.% but was 51 mg.% one month later.
L. T.	54	F	76/60	130/90	28	74	?pulmonary edema	Congestive heart failure IV Aminophyllin induced shock.	Rheumatic Heart Disease. IV Aminophyllin induced shock. Levophed in infusion fluid IV caused pulm. edema to increase. Levophed in oil given with satisfactory response. She died one month later from intractable cardiac failure.
E. V.	68	F	70/60	140/80	32	21	None	Hypertensive CV Dis. with chr. coronary insuff. post-op. shock.	Died 21 hours later. Benadryl potentiated pressor effect when Norepinephrine effect was gradually slipping. At time of death needle out of vein and pressor effect lost before another vein could be cannulated. Emphasizes importance of having good portal for administration.
A. J.	57	M	0/0	235/175	16	12	None	Hypertensive CV disease with Norepinephrine. Hexamethonium B_4 produced shock.	Hypotensive effect of Hexamethonium corrected but Pt. died 5 days later of uremia.
X. I.	56	M	50/20		4	5	None	Arteriosclerotic H. Dis. surgical shock.	Old ant. myocardial infarction. Developed acute hypotension during Surgery. Corrected. Discharged after uneventful P.O. course.
C. C.	21	M	70/60	110/70	24	72	Aggravated Bleeding tendency	Acute Leukemia with hemorrhage	Pt. survived this crisis but died 1 month later.
R. D.	63	M	0/0				None	2°–3° Burns 40% Body surface. RUL pneumonia. Debridement induced shock.	No pressor response. Pt. died.

induced shock.

TABLE 1.—(*Continued*)

Name	*Age*	*Sex*	*BP at Onset*	*Response to Norepinephrine at Onset*	*Amt. of Drug given in mg.*	*Length of Time in Hours*	*Side Reactions to Drug*	*Etiology of Shock*	*Summary and End Result*
C. C.	67	M	0/0	130/100			None	Cor pulmonale with Emphysema. Barbiturate intoxication.	Died after attempting IV Digitalization soon after admission.
E. C.	65	M	0/0	120/100	4	4	None	Recurrent Paroxysmal Auricular Tachycardia with Shock.	Rhythm corrected with Digitalis and Quinidine while BP. sustained with Norepinephrine.
K. C.	46	F	80/60	150/90	4	4	None	Severe Essential Hypertension with Encephalopathy. Hexamethonium Rx.	BP fell from 230/140 to 80/60 with associated toxic symptoms & marked orthostatic hypotension. Hexameth. dc'd 18 hours later symptoms & hypotension persisted. Levophed administration produced immediate remarkable relief of symptoms & BP stabilized later on combination of Hexamethonium & Apresoline.
H. L.	57	F	0/0	90/0	1.7	½	? Vent. Fibrile	Acute anteroseptal myocardial infarction.	Poor response to Levophed from onset. Increased Concentration at 10 min. intervals with only transient effect. Suspect vent. fibrillation occurred terminally.
B. F.	66	M	66/50	130/60	24	24	None	Acute posterolateral myocardial infarction with vent. tachycardia.	Shock and renal shutdown complicated vent. tachycardia treated with Quinidine and then pronestyl. BP sustained by constant IV drip but Pt. received a total of 5000 cc. fluid, IV in face of low urinary output. Death after 24 hours.
H. G.	65	M	60/40	130/100	16	22	None	Acute anteroseptal myocardial infarction.	Pt. died after 22 hours. A total of 4000 cc. fluid given. BP sustained well as long as IV was dripping. Acute hypotension followed administration of IV aminophyllin.
M. X.	39	F	30/0	90/40		20	None	Acute anteroseptal myocardial infarction.	Died after 20 hours. Pressor affect sustained throughout.

TABLE 1.—(*Continued*)

Name	*Age*	*Sex*	*BP at Onset*	*Response to Norepinephrine at Onset*	*Amt. of Drug given in mg.*	*Length of Time in Hours*	*Side Reactions to Drug*	*Etiology of Shock*	*Summary and End Result*
D. X.	59	M	50/20	100/60		60	None	Acute anterior myocardial infarction.	Pt. died of total cardiac failure. BP sustained by intermittent adm. of 4–8 mg. Levophed in 1000 cc. Good pressor response to drug.
L. Z.	63	F	0/0	100/60	24	48	None	Acute anteroseptal myocardial	Levophed started when first seen in E.R. & continued for 48 hrs. At the end of this time BP was stabilized & drug dc'd. Pt. was discharged finally fully recovered.
M. H.	42	F	0/0	140/90	12	12	None	Pulmonary Embolism	Levophed life saving. Shock for 12 hours followed development of Phlebothrombosis and P.E. Subsequently had 3 operative procedures & tolerated each satisfactorily.
A. J.	59	M	60/0	110/70	8	17	None	Pulm. Embolism	Cirrhotic in hosp. for treatment. Life saved by prompt adm. of Levophed.
J. M.	70	M	0/0	150/100				Art. H.D. Resection of colon. 5th P.O. day pulm. embolism.	Pt. survived first episode of shock complicated pulm. embolism. 36 hours later Pt. suffered a second embolism, developed shock again. BP this time was sustained 3 hours with Norepinephrine before Pt. suddenly died.
W. M.	55	M	0/0	140/?	16	18	None	Pulm. Embolism	Pt. had Hypertensive CV Disease, Pulm. emphysema. Pressor effect maintained until death.
K. S.	83	M	60/40	110/70	72	120	None	Pulm. Embolism	Pt. adm. for Perineal Prostatectomy. 12 days P.O. Pt. had sudden transient hemiplegia. Ambulatory & developed acute pulm. embolism and shock. Pressor effect maintained for 3 days with Levophed. The hypotension again developed. Cortisone 50 mg. IM every 12 hours started. Potentiation of pressor effect occurred & BP held at normotensive levels for days when patient suddenly expired. Pulm. emb. with old myocardial infarcts found at postmortem.

instituted and cardiac rhythm had to be judged by frequent auscultation only.

Results. The results of treatment of the individual cases are summarized in Table 1. The final outcome in each case according to the cause of the shock is further demonstrated in Table 2.

Of the total number of 22 patients studied, a definite pressor response bringing the blood pressure out of a shock range was obtained in all but 2 cases. In one of these there was a possibility that norepinephrine may have caused ventricular fibrillation when its concentration was steadily increased in an attempt to obtain a pressor effect. In 7 of these cases, it was thought that the use of the drug had been the single most important factor in saving the patient's life. Two of these had suffered pulmonary emboli; one an acute anteroseptal myocardial infarction; one had severe peripheral circulatory collapse associated with an incompatible blood transfusion; one complicated spinal anaesthesia, and another complicated an episode of refractory auricular tachycardia. The seventh case was an acute hypotensive state associated with the administration of Hexamethonium in the treatment of hypertension.

In 4 of the patients a significant pressor effect was obtained but death occurred later from other causes. Nine died in spite of maintaining the blood pressure levels at a normal range. It is somewhat noteworthy that only one out of 6 cases with acute myocardial infarction survived in spite of a satisfactorily maintained pressor effect in 5.

In the 7 surviving patients the drug was continued for 4 to 74 consecutive hours until it was possible in each case for the patient to maintain his blood pressure in a normal range without the use of drug. One of these (L. T.) had such severe congestive heart failure, complicated by a pulmonary embolus, that administration of norepinephrine by intravenous infusion caused her pulmonary edema to become worse. This was controlled more satisfactorily when the drug was given intramuscularly in the oil base (Levophed-in-oil) and the pressor effect was maintained just as effectively.

Another patient (A. U.) whose shock, due to the hypotensive effect of Hexamethonium, was corrected died 5 days later as a result of uremia. It is thought that the life of another patient (E. V.) might have been saved had measures been taken earlier to insure a continuous means of administering the drug. This patient, who had extreme coronary insufficiency, died after 21 hours when the infusion extravasated and this was not detected soon enough to avoid a rapid fall in blood pressure. The potentiating pressor effect of Benadryl

TABLE 2.—THE FINAL RESULT OF TREATMENT

Etiology	*Life Saving Measure*	*Shock Controlled Death Later—Other Causes*	*Pressor Effect Patient Died*	*No Pressor Response*
Pulmonary Embolism	2	1	2	–
Acute Myocardial Infarction	1	–	4	1
Miscellaneous	4	3	3	1
Total	7	4	9	2

was very dramatically illustrated in this case as is shown in Figure 1.

A hemorrhagic crisis in a patient with acute leukemia precipitated a state of severe shock which was brought under control after 72 hours of administration of norepinephrine, but the patient died one month later of his primary disease. Finally, one patient (V. A.) survived an episode of pulmonary embolization by the immediate use of norepinephrine in combatting the concomitant shock but died 36 hours later as a result of a fatal second embolus.

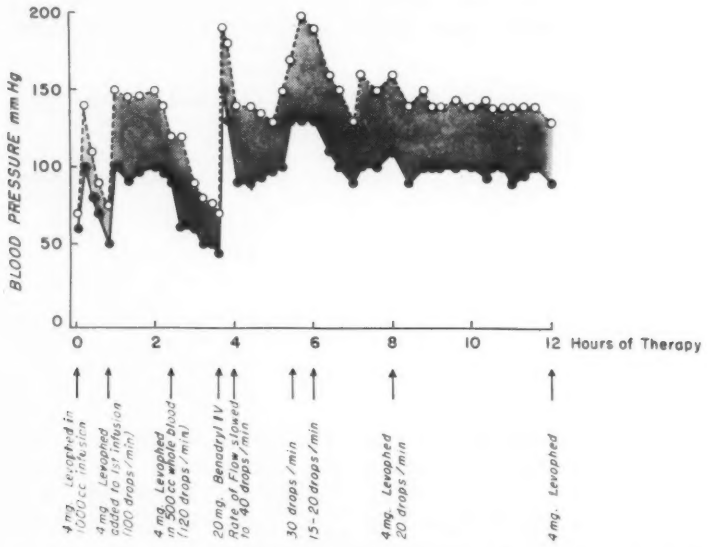

FIG. 1.—Illustration of the potentiating pressor effects of Benadryl on norepinephrine in the case of E. V.

Illustrative Case. A. V., a 59-year-old white man was admitted to the medical service at the V.A. Hospital, Houston, on March 31, 1952 in a semistuporous state. He was known to be a chronic alcoholic and had been diagnosed as having cirrhosis of the liver several years previously. There was a history of sudden onset of left upper quadrant pain, accompanied by shortness of breath and soon followed by nausea and vomiting. Examination revealed the patient to be in a state of peripheral circulatory collapse. The blood pressure was unobtainable and the patient was covered with cold perspiration. The pulse was 120 per minute. A few moist inspiratory rales were heard at the left lung base. The left border of the cardiac dullness was 2 cm. outside the mid-clavicular line. The liver edge was palpable 3 fingerbreadths below the right costal margin but there was no pedal edema.

Laboratory examinations revealed a leukocytosis of 24,400 with a differential count that was essentially normal. An electrocardiographic tracing showed a right axis deviation.

It was strongly suspected that the patient had suffered an acute pulmonary embolus and because of his shock he was immediately started on 4 mg. 1:1000 norepinephrine in a 500 cc. glucose infusion and a continuous intravenous drip was continued for approximately 16 hours at a rate fast enough to maintain satisfactory blood pressure levels. After this time the blood pressure remained fairly stable. These pressor effects are noted in Figure 2. Anticoagulants were not started in view of the uncertainty of liver function. However, on bed rest alone a satisfactory recovery was made and the patient was discharged 3 weeks from the time of admission.

Discussion. Investigative work in the study of "irreversible" shock has centered to some extent on certain vaso-

active materials which appear in the blood under conditions of severe shock and alter the vascular tone of the body[39]. Even before the discovery it was appreciated that in certain forms of shock the critical event seemed obviously to be a collapse of arterial vasomotor tone probably "neurogenic" in origin[35]. In these cases plasma and blood volume remained normal. This situation is well illustrated by the shock state associated with overwhelming infection.

vasomotor apparatus[15,45]. The fact that a pressor response is possible in patients with acute hemorrhagic shock, that was not reversed by blood transfusion alone, suggests that decreased peripheral vascular tone may be an important factor in the shock syndrome, and certain vasoconstrictor agents in conjunction with replacement of blood volume may therefore be of value in its treatment. In these cases an over-all state of vasoconstriction occurs early in an attempt to compensate for the reduced blood volume. In animals it has been shown that terminal failure, when it occurs, is associated with sudden vasomotor collapse and arteriolar dilatation. This apparently occurs in other forms of clinical shock also. However, in other instances, as for example, in a very severe burn, arterial blood pressure may be maintained almost to the point of exitus and the question has been raised whether extreme vasoconstriction may not at times be an unfavorable compensation in shock[35]. This may become of greater considera-

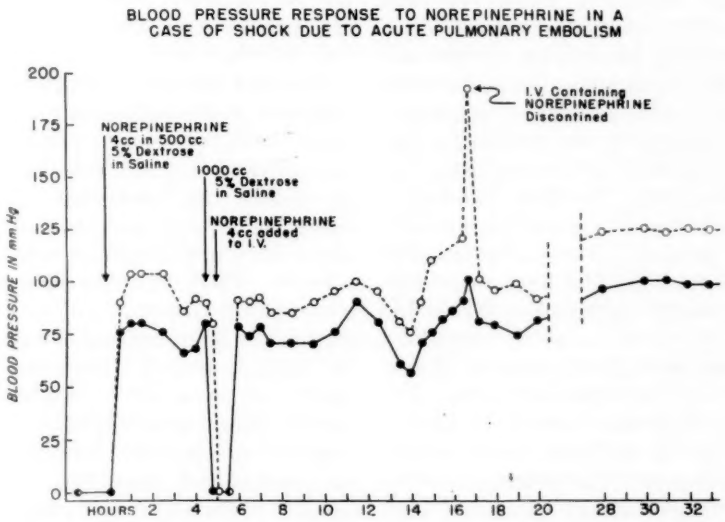
BLOOD PRESSURE RESPONSE TO NOREPINEPHRINE IN A CASE OF SHOCK DUE TO ACUTE PULMONARY EMBOLISM
BLOOD PRESSURE IN mm Hg
200
175
150
125
100
75
50
25
0
HOURS 2 4 6 8 10 12 14 16 18 20 28 30 32
NOREPINEPHRINE 4cc in 500 cc. 5% Dextrose in Saline
1000 cc 5% Dextrose in Saline
NOREPINEPHRINE 4 cc added to I.V.
I.V. Containing NOREPINEPHRINE Discontinued

FIG. 2.—Illustration of the pressor response to norepinephrine in the case of A. V.

Still other, as yet undetected factors may operate in the shock complicating myocardial infarction. Certainly evidence is conflicting on the question of whether intravenous plasma or blood helps or harms the cardiac patient suffering from shock[37]. Experience has shown that if the mortality rate from myocardial infarction is to be appreciably lessened some answer to this particular problem must be found.

Actually, traumatic shock itself frequently has been thought to have an element of faulty performance of the

tion as more experience is gained in the use of such pressor amines as norepinephrine in the treatment of shock. It is an interesting observation that in the case of one of our patients who had extensive burns (R. O.) we were unable to obtain any pressor effect when norepinephrine was administered.

Early in this century Barger and Dale[3] proposed the name "sympathomimetic" for substances that simulate the effect of the sympathetic nerves and mediate various physiologic responses. As late as 1939, it was believed that Adrenalin alone was the mediator of the adrenergic nerves[43] but with increasing knowledge of the action of the sympathomimetic amines the idea gradually developed that norepinephrine might be responsible for the excitatory reaction and epinephrine for the inhibitory ones. In 1946, Von Euler found in animals a norepinephrine-like substance present in various tissues and organs containing adrenergic fibers[44]. Then the recent chemical isolation by Tainter[42] of the l-isomer of norepinephrine from the racemic mixture of the drug led to the demonstration by Luduena[25] that this isomer is physiologically 27 to 60 times more active than the d-isomer. The subsequent detection of this substance in natural lots of Adrenalin[42] and in extracts of pheochromocytomas[16] stimulated a renewal of interest in its pharmacologic and hemodynamic actions especially as they might relate to the problem of arterial hypertension. Its synthesis has opened up a partially new approach to the problem of treating acute hypotension.

The total effect of norepinephrine is one of general vasoconstriction with little or no change in the cardiac output as determined by right heart catheterization[15]. Barcroft and Starr[2] compared the effects on the cardiac output by using the ballistocardiograph and found actually some decrease in the cardiac output with norepinephrine. A few discrepancies in the methods used was thought to explain some of this difference in results, however.

It is postulated that the 2 factors concerned in the production of the bradycardia by norepinephrine are either a direct action on the pacemaker or a reflex inhibitory action secondary to its vascular action. More likely it is the former explanation since it can be abolished by giving atropine[41]. Norepinephrine has been found experimentally to increase coronary blood flow and produce coronary vasodilatation[5,-12,15,28,40,43].

Lee and Melville have shown experimentally that norepinephrine may induce both coronary vasoconstriction and dilatation and that the coronary blood flow per heart beat is generally decreased except with very large doses when coronary dilatation was found to occur[23]. They thought that the different results reported in the literature may have been related to differences in dosage used by previous investigators. It was also shown in their studies that norepinephrine produced marked myocardial stimulation and they suggested that this effect was largely responsible for mechanically impeding coronary flow. This stimulation is followed subsequently by an intense and prolonged increase in rate of coronary flow due to coronary dilatation. They postulated that the dilator effects may be due to metabolites produced during the myocardial stimulation induced by the drug. Because of these actions of the drug on the heart it was thought conceivable that they might also play an important role in the blood pressure responses.

Goldenberg believes that the moderate increase in cardiac output and consequent lowering of the peripheral resistance after a prolonged period of norepinephrine infusion may be

ascribed to the release of endogenous epinephrine in responses to the prolonged procedure and accompanying anxiety of the patient.

The report of Nathanson[31] supports the observation of Goldenberg who found no significant cardiac stimulation. Ten patients with complete heart block were given norepinephrine in doses which produced definite pressor effects and only minimal and transient increases in ventricular rate were observed while epinephrine produced a marked and sustained increase. These authors believe that norepinephrine might be considered a very desirable pressor drug in the treatment of the shock of coronary occlusion. They do mention in connection, however, the work of Garb[14]. The latter has shown in experimental animals whose hearts have been sensitized by hydrocarbon inhalation (cyclopropane, chloroform, ethylchloride, or benzine) that the administration of norepinephrine consistently induced ventricular fibrillation. Because the ischemic myocardium is also predisposed to this serious type of arrhythmia, it would appear that there might be a definite hazard in the use of this agent in conditions in which the coronary circulation is inadequate. In this respect norepinephrine is apparently similar to epinephrine for the incompatibility of this latter drug and cyclopropane is quite familiar to the anesthesiologist. In some unknown way cyclopropane sensitizes the ventricular ectopic centers only to those sympathomimetic amines with the catechol ring structure. In the experimental studies by Garb, a characteristic electrocardiographic pattern was noted just before the onset of ventricular fibrillation. This consisted of progressive lowering of the voltage of the QRS complex with higher voltage in the T wave than in the QRS complex.

Peripheral vasoconstriction due either to sympathomimetic drugs or to sympathetic nerve activity is known to depress renal blood flow[7]. Moyer has reported the renal effect of administering a continuous infusion of either norepinephrine or epinephrine[30]. Renal blood flow decreased and the filtration fraction increased due to constriction of the efferent arteriole. Later, as the infusion rate was increased the afferent arteriole shared in the increased vasoconstriction and a significant number of glomeruli may be eliminated from the renal circulation as shown by a proportional decrease in renal plasma flow, glomerular filtration rate, and maximum tubular absorption of glucose. This effect could be blocked by adrenergic blocking agents. There was no evidence to support the hypothesis that epinephrine or norepinephrine activate renal vascular shunts in dogs. Churchill-Davidson[10] reports the same findings in both unanesthetized and anesthetized man. There is no information as yet available on the effect of these drugs on the renal circulation in cases of shock due to hemorrhage and reflex peripheral vasodilatation, or a fall in cardiac output. Certainly there is no reason to suppose that the reaction would be more favorable.

Norepinephrine does not produce central nervous system stimulation with the associated anxiety, discomfort, and peculiar feelings that epinephrine does. It is also 8 times less toxic. The pressor effects sometimes reported with epinephrine may be due to the fact that "natural adrenalin" on the market may contain up to 20% norepinephrine[43].

In reviewing the clinical literature occasional comments are encountered to the effect that increasing amounts of norepinephrine are often required after it has been continuously administered over several hours. This phenomenon of tachyphylaxis is well known to the pharmacologist. We have noted this phenomenon in the clinical use of norepinephrine also and conse-

quently the work which has appeared in the experimental literature regarding the potentiating effect of various substances, especially the adrenal cortical hormones on the action of norepinephrine has been of great interest to us. Progressive insensitivity to norepinephrine has been noted in adrenalectomized dogs subjected to stress[21]. Single injections of adrenocortical extract potentiated the pressor effects to previously ineffective concentrations of norepinephrine but not in the normal control. Three patients were also observed by Kurland to have a striking potentiation of the blood pressure response when given cortisone or ACTH within 24 hours after starting these drugs. The effect disappeared within 36 hours after omission of these agents[21]. Similar observations have been reported by Pamey[32]. Apparently the 11-oxysteroids restore normal reactivity of the blood vessels to both endogenous and exogenous norepinephrine and make possible effective circulatory adjustments in response to the needs of the organism during increased vascular demands in the periphery. Fritz further studied the factors responsible for peripheral circulatory failure exhibited by adrenalectomized animals subjected to stress[13]. Under direct visualization he observed meso-appendiceal vessels for sensitivity to various agents both in normal and adrenalectomized rats. In essence his findings seem to indicate that the cortical steroids are necessary to allow blood vessels to respond regularly and repeatedly to minute amounts of norepinephrine, and, at the same time, to prevent the toxic effects upon blood vessels of large amounts of norepinephrine.

It has been suggested by Raab that the potentiation of sympathomimetic pressor effects under the influence of exaggerated adrenocortical activity may be a pathogenic factor in essential hypertension[33]. More recently he and his co-workers have presented evidence that the presence of a certain amount of sodium in the body is necessary to maintain full cardiovascular pressor responsiveness to both norepinephrine and desoxycorticosterone[34]. The latter intensifies the pressor effectiveness of norepinephrine during normal salt intake and weakens the response on salt withdrawal. Sodium seems to act then as a sort of physiochemical mediator of the pressor effect.

The antihistamines have been found to enhance the response to both epinephrine and norepinephrine. Again the mode of action is not clearly understood. It has been postulated that pyribenzamine might inhibit amine oxidase or tyrosinase[46]. Haley has found that these drugs have a vasoconstrictor action on the precapillary sphincters of the mammalian capillary bed[32]. Others have suggested the blocking of vasodepressor receptors as a possible mode of action[8] or that the antihistamine drugs diminish or block vagal cardioinhibitory impulses. The rather profound response to a single injection of 20 mg. of Benadryl intravenously in one of our patients (E.V.) is illustrated in Fig. 1. Further observations using these norepinephrine potentiators is being carried out and will be reported at a later date.

Since the synthesis of norepinephrine it has been used extensively in the treatment of acute hypotensive states from various causes. Cappe and Pallin has reported its successful use along with transfusions in 3 cases of massive obstetric hemorrhage[6]. A number of reports have appeared in regard to its success in the maintenance of blood pressure during and following thoracolumbar sympathectomy[9,11,20,24]. The same is also true for the operative and postoperative management of cases with a pheochromocytoma. The facility with which norepinephrine can be

used to maintain the blood pressure during spinal anesthesia, especially when methedrine fails to sustain a pressor response, has been reported[9]. Luger has successfully treated a case of profound shock in a 72-year old man who had suffered a perforation of a gastric ulcer and subsequently was able to withstand a surgical resection because of the pressor effects of norepinephrine[26].

Very little has been reported on the clinical use of norepinephrine in the control of shock complicating certain cardiovascular situations such as acute myocardial infarction or pulmonary embolism. Because of the drug's immediate action, short duration of action, low toxicity, ease of administration and control, elevation of both systolic and diastolic blood pressure without significant change in cardiac output, reported absence of coronary constriction, and because its action arises from and is mediated by normal physiologic mechanisms it has been thought that it may fulfill some of the theoretic requirements at least for combating cardiogenic shock. Whether or not this impression will remain valid can be answered only from further experience with its use in such conditions. The tendency for it to produce increased myocardial irritability may prove to be a limiting factor in the usage.

Miller and Baker describe the results of its use in the treatment of shock due to acute myocardial infarction[29]. Norepinephrine was given by intravenous infusion to 7 patients in profound shock in their series. Significant blood pressure elevations were attained in 4 of these. In no instance was there any untoward effect noted on the prevailing cardiac rhythm, the state of congestive failure or the subjective status of the patient. However, only one patient in the group survived his infarction. This compares with our experience using norepinephrine in the shock due to myocardial infarction. Certainly no conclusion can be drawn in regard to the bearing it had on ultimate mortality.

Even more recently, Kurland and Malach report a significant pressor response in 12 of 17 courses of treatment in 14 patients with shock accompanying acute myocardial infarction[22]. Four of these patients ultimately survived. Five died in one to 41 days after recovery from the initial episode of collapse.

Even with such small series it appears that other factors have yet to be recognized and effectively managed before an appreciable lowering of the mortality in these cases can be attained. Wiggers has brought forward evidence that there is a true failure of the myocardium in irreversible shock in dogs[45]. Certainly, however, measures to aid myocardial function in shock, such as digitalization, have been ineffective in both animals and man[36].

Seemingly the results of treatment of shock other than cardiogenic in origin with norepinephrine is more encouraging.

In the clinical use of norepinephrine one can at all times during its administration carefully control and regulate the pressor effect by attention to the rate of drip and the height of the blood pressure. In all cases in which it is contemplated that norepinephrine will require continuous intravenous administration over several days it might be well to insert and advance a small polyethylene tube well into a vein and secure it in place as suggested by Greenwald[18]. This author reports 2 cases out of 5 who inadvertently had infiltration of the subcutaneous tissues with norepinephrine containing solution developed extensive tissue necrosis within 48 hours. This was thought to be solely due to the local vasoconstrictor effect of the drug. We have encountered similar instances of difficulty because of failure to insure the patency of venous

channels during its administration. When extravasation occurs it is often difficult to find another suitable vein due to the circulatory status of the patient at that time. The pressor effects are rapidly dissipated with temporary discontinuance of the norepinephrine. Because norepinephrine is unstable it is kept in an ampule of either ascorbic acid or 0.1N hydrochloric acid and added to the infusion solution just before use. Generally 4.0 cc. of 1:1000 solution (4 mg.) is added to 500 cc. or 1000 cc. of glucose or normal saline. It is well to remember that hypertensive patients may be more sensitive than normotensive individuals and smaller doses may maintain a constant level. Larger doses seem to be required after hexamethonium compounds have been used. Emphasis must always be placed on the fact that the only satisfactory guide to dosage is in the observation of the effect produced. The main danger in excess dosage is the production of cerebral hemorrhage but this should not occur with adequate vigilance to the height of the blood pressure which can always be lowered promptly by slowing the rate of the drip. Experimentally the commonest result of administration of toxic doses is the production of pulmonary edema and adrenergic blocking agents, such as dibenamine may prevent this from occurring[27]. Because of the increase in arterial, capillary, and venous pulmonary pressures following the administration of norepinephrine to normotensive human beings[17], the possibility of aggravating or producing pulmonary edema after its use in patients with myocardial infarction must be considered. Caution should be exercised at least in those patients already in congestive heart failure or those with mitral stenosis.

From this discussion one can postulate, to some extent, those situations in which the use of norepinephrine might be beneficial. A few of these situations have been mentioned above. One might also expect it to be of help in patients with severe hypertension or coronary artery disease to prevent dangerous drops in blood pressure during surgery; in any situation characterized by a sudden massive hemorrhage; in cardiac surgery especially when procaine (a vasodilator) is used to reduce cardiac irritability; following the use of any of the hypotensive drugs including hexamethonium, hydrazinophtalazine, or veriloid; and in the shock state associated with overwhelming infection. It appears also from experience being gained at the present that this may extend to include the shock complicating myocardial infarction or pulmonary embolism, lower nephron nephrosis, or the administration of a mismatched whole blood transfusion.

Summary and Conclusions. 1. An attempt has been made to present the rationale for therapy of acute hypotensive states with pressor amines such as norepinephrine.

2. The results of treatment in a series of 22 patients is reported; shock was associated in 5 with acute pulmonary embolism, in 6 with acute myocardial infarction and from miscellaneous causes in 11. The outcome was poorest in those cases suffering with so-called "cardiogenic shock."

3. The pharmacologic and hemodynamic effects of norepinephrine was reviewed.

4. Mention has been made of the interesting and possibly highly significant potentiating pressor effects of adrenal cortical steroids and the antihistamines.

REFERENCES

1. **Baco, Z. M.:** J. Pharmacol. & Exp. Therap., **95** (Part II), 1, 1949.
2. **Barcroft, H., and Starr, I.:** Clin. Science, **10,** 295, 1951.
3. **Barger, G., and Dale, H. H.:** J. Physiol., **32,** 401, 1905.
4. **Blaschho, H.:** Ibid., **101,** 337, 1942.
5. **Burn, J. H., and Hutcheon, D. E.:** Brit. J. Phar., **4,** 373, 1949.
6. **Cappe, B. E., and Pallin, T. M.:** Anesthiol., **12,** 728, 1951.
7. **Chasis, H., Ranges, H., Goldring, W., and Smith, H.:** J. Clin. Invest., **17,** 683, 1938.
8. **Chen, G., and Russell, D.:** Fed. Proc., **8,** 280, 1949.
9. **Churchill-Davidson, H. C.:** Brit. Med. J., **2,** 1551, 1951.
10. **Churchill-Davidson, H. C., Wylie, W. D., and Miles, B. E.:** Lancet, **2,** 803, 1951.
11. **Deterling, R. A., and Apgor, V.:** Ann. Surg., **133,** 37, 1951.
12. **Folkow, B., Frost, J., and Uvnäs, B.:** Acta physiol. scandinav., **17,** 201, 1949.
13. **Fritz, I., and Levine, R.:** Am. J. Physiol., **165,** 456, 1951.
14. **Gofman, J. W., Lindgren, F. T., and Elliott, H.:** J. Biol. Chem., **179,** 973, 1949.
15. **Goldenberg, M., Apgor, V., Deterling, R., and Pines, K. L.:** J. Am. Med. Assn., **140,** 776, 1949.
16. **Goldenberg, M., Faber, M., Alston, E. J., and Chargaff, E. C.:** Science, **109,** 534, 1949.
17. **Goldenberg, M., Pines, K. L., Baldwin, E. de F., Greene, D. G., and Roh, C. E.:** Am. J. Med., **5,** 792, 1948.
18. **Greenwald, H. P., Gootnick, A., Luger, N. M., and King, J. A.:** New Eng. J. Med., **246,** 252, 1952.
19. **Haley, T. J., and Harris, H.:** J. Pharmacol. & Exp. Therap., **95,** 292, 1949.
20. **Judson, W. E., Epstein, F. H., and Wilkins, R.:** J. Clin. Invest., **29,** 1414, 1950.
21. **Kurland, G., and Friedberg, A. S.:** Proc. Soc. Exp. Biol. & Med., **78,** 28, 1951.
22. **Kurland, G. S., and Malach, M.:** New Eng. J. Med., **247,** 383, 1952.
23. **Lee, F. C., and Melville, K. I.:** J. Physiol., **113,** 365, 1951.
24. **Loofbouraw, D., and Palmer, R. S.:** New Eng. J. Med., **243,** 295, 1950.
25. **Luduena, F. P., Ananenko, E., Siegmund, O. H., and Miller, L. C.:** J. Pharmacol. & Exp. Therap., **95,** 155, 1949.
26. **Luger, N. M., Kleinman, A., and Fremont, R. E.:** J. Am. Med. Assn., **146,** 1592, 1951.
27. **MacKay, E. M., and Pecka, E. F., Jr.:** Proc. Soc. Exp. Biol. & Med., **75,** 245, 1950.
28. **March, D. F., Pelletier, M. H., and Ross, C. A.:** J. Pharmacol. & Exp. Therap., **92,** 108, 1948.
29. **Miller, A. J., and Baker, L. A.:** Arch. Int. Med., **89,** 591, 1952.
30. **Mover, J. H., and Handley, C. A.:** Circulation, **5,** 91, 1952.
31. **Nathanson, M. H., and Miller, H.:** Am. Heart J., **40,** 374, 1950.
32. **Pamey, E. R., Goldstein, M. S., and Levine, R.:** Am. J. Physiol., **165,** 450, 1951.
33. **Raab, W., Humphreys, R. J., and Lepeschkin, E.:** J. Clin. Invest., **29,** 1397, 1950.
34. **Raab, W., Humphreys, R. J., Makous, N., DeGrandpré, R., and Gigge, W.:** Circulation, **6,** 373, 1952.
35. **Richards, D. W.:** The Merck Report, April, 1952, p. 18.
36. **Richards, D. W., Jr.:** Bull. New York Acad. Med., **20,** 363, 1944.
37. **Sampson, J. J., and Singer, I. M.:** Am. Heart J., **38,** 54, 1949.
38. **Sherrod, T. R., Loew, E. K., and Schlaemer, H. F. I.:** J. Pharmacol. & Exp. Therap., 89, 247, 1947.
39. **Shorr, E., Zweifach, B. W., Furchgott, R. F., and Baez, S.:** Circulation, **3,** 42, 1951.
40. **Smith, D. J., and Coxe, J. W.:** Proc. Soc. Exp. Biol. & Med., **75,** 245, 1950.
41. **Sutton, G. C., Kapper, A., and Reale, A.:** J. Lab. & Clin. Med., **36,** 40, 1950.
42. **Tainter, M. L., Tullar, B. F., and Luduena, F. P.:** Science, **107,** 39, 1948.
43. **Von Euler, U. S.:** Brit. Med. J., **1,** 105, 1951.
44. Idem: Acta physiol. scandinav., **12,** 73, 1946.
45. **Wiggers, C. J.:** Am. Heart J., **33,** 633, 1947.
46. **Yoakman, F. E., Chess, D., Hays, H. W., Rennick, B., and Moyer, R.:** Fed. Proc., **5,** 216, 1946.

TRANSBRONCHIAL INSTILLATION OF RADIOACTIVE GOLD COLLOID IN THE LUNG OF THE DOG. DISTRIBUTION STUDIES, SURVIVAL AND PATHOLOGY*

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CARCINOMA of the lung is of growing clinical importance and in many instances is refractory to conventional therapeutic approaches. Seventy per cent of patients are inoperable when first seen[2] and external irradiation usually produces disappointing results. It is urgent to devise new methods of attack in view of the high incidence of this disorder[2,9]. No method of localization of radioisotopes in lung tumors by "metabolic" means is yet known.

The experiments reported here were designed to localize a radioisotope in the lung and the regional lymph nodes by exploitation of the physiology of pulmonary lymphatics in relation to colloidal material. Previous experiments have shown that water instilled in the lung of the dog disappears rapidly; actually, with a half-time of less than 4 minutes[8]. Thus, when a colloidal solution is introduced, the water would disappear rapidly, leaving the solid on the alveolar and possibly also on the bronchial epithelial surface. Our own studies[7] and those of Courtice[1] show that little if any colloidal material passes from the alveolus to the pulmonary capillary blood directly.

This presentation reports the distribution of radioactive colloidal gold solutions instilled into the lung via the tracheobronchial route, the pathological alterations produced by the resulting internal radiation and survival following various dosages.

Methods. Dogs were anesthetized with Nembutal, laid upon the right side and remained on the right side until recovery from the anesthetic. They were kept in clean, comfortable quarters and fed a wholesome kennel diet but received no supportive therapy.

In the first 6 experiments a catheter with a balloon was inserted in the trachea and the balloon inflated to prevent reflux. Radioactive colloidal gold solution** was diluted appropriately with water in such a manner that the dose administered would be contained in 0.5 cc. per kg. of dog. The measured solution was poured down the tracheal tube. Two dogs were sacrificed at 17 hours, one at 24 hours, one at 6 days and one at 15 days. The

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** $Gold_{198}$ was produced by an $n\gamma$ reaction in the pile at Oak Ridge and prepared in colform at Abbott Laboratories, North Chicago, Ill., by the method devised by Hahn[3].

2 dogs sacrificed at 17 hours received only tracer quantities of gold and no radiation effects were anticipated. The remainder received amounts of the isotope which produced moderate to high levels of internal radiation. Organs from these animals were removed at autopsy, weighed, blocks of tissue reserved for histopathological examination, and a weighed aliquot prepared for radioactivity assay by dry ashing. The ash was dissolved in aqua regia, reduced to dryness, redissolved in water, transferred to counting cups and counted with a thin mica window counting tube. Counting data were expressed in terms of activity per gram of organ. These data were converted to relative activity by taking, arbitrarily, the highest lung lobe activity per gram as 100 and expressing the other tissue activities as a percentage thereof. Since the distribution of the colloid is of no interest beyond the time it gives consequential tissue irradiation, distribution studies were confined to the first 2 weeks during which period 98% of the irradiation would be delivered.

In 18 dogs the material, prepared in the same manner, was introduced into the intermediate lobe of the right lung by bronchoscopy and instillation through a long 18-gauge stainless steel tube with a hypodermic syringe fitting affixed to the external end*. No further distribution studies were done. Data obtained concerned survival and gross and microscopic examination of autopsy material.

Results. a) DISTRIBUTION STUDIES. Table 1 shows the distribution of radioactive colloidal gold following instillation in the trachea in 6 dogs. Previous experience has shown that gold colloid of the sort employed here, with a particle diameter very close to 0.05 micron, introduced into the circulation is rapidly and almost completely removed by the liver and to a lesser extent by the spleen. Very low levels of activity in the liver and spleen samples show that this colloid, when introduced into the alveoli via the tracheobronchial route does not enter the blood vascular system in any consequential amount.

The highest levels of activity are confined to the right lung. Apparently the colloid is fixed early, since none is spread to the contralateral side which would be inevitable were the material free when the dog regained conscious-

TABLE 1.—DISTRIBUTION OF RADIOGOLD COLLOID AFTER TRACHEAL INSTILLATION INTO ANESTHETIZED DOGS IN THE RIGHT LATERAL POSITION. RELATIVE ACTIVITY PER GRAM OF TISSUE WITH SITE OF HIGHEST ACTIVITY TAKEN AS 100

		Time After Instillation					
	Organ	*17H*	*17H*	*24H*	*4D*	*6D*	*15D*
R.	Lobes of Lung						
	Apical	59	100	100	53	100	93
	Cardiac	100	65	0.5	100	—	68
	Intermediate	—	—	—	44	47	100
	Diaphragmatic	46	78	29	16	44	12
R.	Hilar Lymph Node	11	43?	2.9	1.0	6.5	31
L.	Lobes of Lung						
	Apical	1.5	0.1	0.2	0.1	0.6	0.1
	Cardiac	3.1	—	0.3	0.1	0.1	—
	Diaphragmatic	0.8	0.1	0.3	0.1	1.2	0.1
L.	Hilar Lymph Node	3.0	2.2	0.1	0.1	0.1	0.5
	Mediastinal Node	0.8	0.7	0.4	2.4	0.6	9.0
	Liver	0.2	0.1	0.1	0.1	0.1	0.1
	Spleen	0.5	0.2	0.1	0.1	0.1	—

? Contamination by technical error.

*These tubes were provided through the courtesy of Mr. L. E. Eisele, President, and Mr. H. C. Crump, Manager, Eisele Co., Nashville, Tenn.

ness. This is a tribute to the efficacy of the right lateral position in preventing contralateral spread of liquid. By the second week, appreciable amounts are found in nodes from the mediastinal region.

b) SURVIVAL FOLLOWING VARIOUS DOSES. In Table 2 are presented data relevant to survival or time of sacrifice.

c) PATHOLOGICAL CHANGES. The actual dosage by trachea instillation in the dogs sacrificed at 24 hours, 4 days and 6 days was about 0.7 mc. per kg. of body weight. The dog sacrificed at 15 days received 3 times as much, 2 mc. per kg. An estimate, based upon some rather sweeping assumptions, indicates the right lung of this dog received as much as 17,000 roentgens. Only this lung showed striking gross changes, with adherent fibrinous pleuritis and hemorrhagic lobular consolidation. Microscopically there was extensive necrosis accompanied by dense fibrin precipitation and hemorrhage as well as local capillary thrombosis. Marginal zones about the necrotic area showed exudation of monocytes and polymorphonuclear leukocytes, and, more distantly, there was slight edema and mononuclear infiltration. The left lung was essentially normal both in the gross

TABLE 2.—SURVIVAL OR TIME OF SACRIFICE AFTER TRACHEOBRONCHIAL INSTILLATION OF VARIOUS DOSES OF GOLD$_{198}$ IN THE LUNG

Dose per Kg.	*Dog. No.*	*Survival*
0.5 mc.	24	Died, 1 week
	9	Sacrificed, 2 weeks
	30	*Died, 3½ weeks
	12	Sacrificed, 4 weeks
	14	Sacrificed, 6 weeks
	15	Sacrificed, 6 weeks
	26	Sacrificed, 10 weeks
	27	Sacrificed, 12 weeks
0.66 mc.	3	Sacrificed, 1 day
	5	Sacrificed, 4 days
	6	Sacrificed, 6 days
	10	Sacrificed, 2 weeks
	13	Sacrificed, 4 weeks
	19	Sacrificed, 6 weeks
1.0 mc.	11	Sacrificed, 2 weeks
	20	*Died, 3 weeks
	22	*Died, 3 weeks
2.0 mc.	7	Sacrificed, 15 days
4.0 mc.	16	*Died, 3 weeks
6.0 mc.	17	*Died, 3 weeks
10.0 mc.	18	*Died, 1 week

* Death apparently due to or secondary to irradiation effects.

Survival after doses of 1 mc. or more per kg. is significantly ($P<.05$) poor. Doses of 0.66 mc. and 0.50 mc. per kg. were fairly well tolerated. One dog died one week after 0.50 mc. per kg., but this was probably not due to irradiation effects. The other dog which died 3½ weeks after 0.50 mc. per kg. exhibited a small shelled-out lung abscess in the treated lobe which probably contributed to his demise, although other dogs with even greater gross lesions survived long periods of time before being sacrified.

and microscopically. The bone marrow was not affected.

Changes in the lungs in the 24-hour, the 4-day and the 6-day dogs receiving much lower dosage, perhaps 5,000 roentgens, were different. At 24 hours, in those lobes of highest dosage, there was a mild acute non-necrotizing pneumonitis, some intraalveolar edema and mild polymorphonuclear infiltration. The most affected lobe showed acute edema of the pleura and the subpleural interstices. The left lung and the less affected parts of the right lung showed patches of atelectasis alternating with acute emphysema resembling Liebow, Warren and De Coursey's[6] description of changes in Japanese radiation casualties who died in a few days. Since radioactivity in these tissues was low, we must ascribe these changes to gamma radiation from the contralateral gold source.

At 4 days, the disease process in sites of highest activity was less notable and consisted in the main of areas of alternating atelectasis and acute emphysema, present also, but to a lesser degree, in lobes of low activity. Collapsed areas contained considerable numbers of phagocytic mononuclear cells. By the end of 6 days there was very little change of any kind in the lungs and pleura. There were small areas of partial collapse in all sections and slight emphysema in areas of high dosage.

Lymph nodes, at 24 hours, which exhibited but little activity nevertheless showed striking changes. There was conspicuous dilatation of the sinusoids and the lumina contained numbers of polymorphonuclear cells and erythrocytes. Also very notable was pronounced erythrophagocytosis on the part of large mononuclear cells. The follicles and cortex of these low-activity nodes were essentially normal. The nodes which showed higher radioactivity exhibited poorly defined follicles and small areas of cortical necrosis and disorganization.

Later, at 4 days, the nodes in the regions proximal to the affected lung showed loss of well-defined follicles and areas of loss of lymphocytes from the cortex. There was a relative increase in the reticulum cells in the cortex and of mononuclear phagocytes in the sinusoids. There were a few mitoses among the reticulum cells. Changes in the more remote nodes were similar but slight.

At 6 days the most active nodes were devoid of follicles. There was a marked increase in reticulum cells in the cortex at the expense of the lymphocytes. Scattered monocytes contained hemosiderin. The cortices were depleted of all normal cells but there were moderate numbers of mitotic figures among the reticulum cells. Less affected, more remote nodes showed changes which were similar except in degree. There were occasional foci of large cells which appeared to represent the remnants of follicles.

At 15 days, the changes were more advanced but with evidence of regeneration in the form of pale hyperplastic follicles capped with crescents of lymphocytes. The sinusoids were dilated and contained many phagocytes filled with pigment and debris. The less affected nodes showed more numerous follicles and cortical lymphocytes.

In the animals receiving from 0.5 to 1.0 mc. per kg. of the radiogold colloid by bronchoscopic instillation in the intermediate lobe of the right lung, the local pathological process was much more severe than that described in the animals above where the dose was more diffusely distributed. This is attributable to the fact that the roentgen dosage to the single treated lobe was greater by a factor of approximately 4 when the radioactive material was concentrated in a single lobe. The characteristic local lesion in the treated lobe

was an area of necrosis of greater or less extent usually with central softening or even an abscess cavity. There were usually evident, in the gross, several concentric zones, an outer hemorrhagic consolidation, within this a zone of grayish consolidation and necrosis and a central zone of greenish necrosis which in some instances as mentioned above had shelled out to produce an abscess cavity with a shaggy wall. The largest abscess cavity was observed to be 3 cm. in diameter. The untreated lobes were in general normal in gross appearance with the exception of recent pneumonitis in 2 or 3 instances.

On microscopic examination, changes were seen which related more closely to the time which had elapsed since treatment than to the variation in dosage from 0.5 to 1.0 mc. per kg. After 2 weeks, the changes seen in lymph nodes represented varying degrees of injury and repair, depending upon the amount of time which had elapsed and the degree to which the colloid had drained to the node.

At higher dosage level, where death occurred in from 1 to 3 weeks, extensive hemorrhagic pneumonitis and hemorrhagic pleural exudation were present. These dogs were discarded from further consideration.

Discussion. The results of these experiments may be viewed in two ways. As an investigation of the manner in which inert colloidal particles of about 0.05-micron size are handled by the lung when introduced into the alveoli, the results are informative. As experiments designed to lay the ground work for an attack on carcinoma of the lung they are disappointing.

It is of interest that the lateral position so effectively localizes liquid in the dependent lung. In many clinical situations it is desirable to prevent contralateral spread, especially of infected material, and the data reported here reinforce existing confidence in the lateral position for this purpose at least for short periods of time.

The colloid is evidently rapidly fixed, presumably first by absorption on the alveolar wall and subsequently possibly by phagocytosis. Very little enters the blood stream by any route, since activity of spleen and liver samples is low and the reticulo-endothelial system of these organs is highly efficient at removing this colloid from the blood stream[3]. It is evident from the distribution studies that the bulk of the gold stays in the lung into which it is introduced. Small amounts move to nearby lymph nodes in a few days and to the more remote mediastinal nodes in a week or two. This is in sharp contrast to the behavior of silver colloids[5] and silver coated gold colloids[4].

For cancer therapy there are two disadvantages to the instillation of gold colloid in this manner. The lung where it lodges is too heavily irradiated while the regional lymph nodes, which are the common metastatic site, receive only trivial amounts of radiation since the activity of the gold is largely spent before it arrives there. While the use of a longer-lived isotope in inert colloidal form might help surmount this latter difficulty, it could hardly be expected to ameliorate the former. This consideration led to a change in attack on the problem rather than a change to an isotope of longer half-life[4].

Summary. 1. When radioactive colloidal gold solution with particles of approximately 0.05 micron in diameter is instilled into the lung or one of its lobes via the tracheobronchial route, the colloidal material is rapidly fixed in the alveoli.

2. In a few days some of the material makes its way via lymphatics to nearby lymph nodes and by 2 weeks a considerable amount may have progressed to the mediastinal nodes.

3. Little if any of the colloid enters the blood stream as evidenced by failure of consequential amounts to accumulate in the liver and spleen.

4. The lung tissue in the neighborhood of the fixed colloid is heavily irradiated but due to the short half-life of $gold_{198}$ and its slow progress via lymphatics, the regional lymph nodes are not heavily irradiated. The prospect of using this particular technique in therapy of cancer of the lung is, therefore, not encouraging.

5. Survival data indicate that doses in one lobe of the lung of 0.7 mc. per kg. or less are fairly well tolerated in the dog, while doses of 1.0 mc. per kg. and above are poorly tolerated.

6. The morbid anatomy of the lesions observed is described.

REFERENCES

1. **Courtice, F. C.,** and **Simmonds, W. J.**: J. Physiol., **109,** 103, 1949.
2. **Graham, E. A.**: Surg. Clin. N. Am., **30,** 1259, 1950.
3. **Hahn, P. F.**: A Manual of Radioisotope Therapy. New York, N. Y.: Academic Press, 1951.
4. **Hahn, P. F.,** and **Carothers, E. L.**: Brit. J. Cancer, **5,** 400, 1951.
5. **Hahn, P. F., Rouser, G., Brummitt, H., Moorehead, J.,** and **Carothers, E. L.**: J. Lab. & Clin. Med., **39,** 624, 1952.
6. **Liebow, A. A., Warren, S.,** and **De Coursey, E.**: Am. J. Pathol., **25,** 853, 1949.
7. **Meneely, G. R., Kory, R. C., Auerbach, S. H.,** and **Hahn, P. F.**: Fed. Proc., **10,** 365, 1951.
8. **Meneely, G. R., Qualls, G.,** and **Curtis, H. J.**: Proc. Am. Physiol. Soc., Am. J. Physiol., **167,** 809, 1951.
9. **Ochsner, A.,** and **De Bakey, M.**: Surgery, **8,** 992, 1940.

METABOLIC, ISOTOPIC, AND PATHOLOGICAL DIFFERENCES BETWEEN SODIUM-5-IODO-2-THIOURACIL AND THIOURACIL PLUS POTASSIUM IODIDE*†

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The clinical experience[3,7,8,9,17], with Sodium-5-Iodo-2-Thiouracil, henceforth called Itrumil, has shown that this compound has an effective anti-thyroid action and no clinical goitrogenicity. The rapid clinical response obtained with Itrumil has raised the question whether or not the mode of action of this drug is not primarily an iodine effect. In order to clarify this question, a study with equimolar quantities of thiouracil and iodide was undertaken and is the objective of this report.

Methods and Materials. Forty-eight unselected patients with thyrotoxicosis received treatment as follows: Thirty-seven patients received 40 courses of treatment with Itrumil in daily doses from 50 to 600 mg. for a period of 13 to 365 days. The studies in 15 of these cases have been reported earlier[3]. Eleven patients received 12 courses of treatment with an admixture of thiouracil and potassium iodide prepared in the form of capsules. Each capsule contained the molecular equivalent of 200 mg. Itrumil, (100 mg. of thiouracil and 131 mg. of potassium iodide). The daily dose given to these patients ranged from 200 to 800 mg. for a period of 21 to 140 days.

The maximal clinical response, histological comparison and physiological findings are reported herein.

The sex and age distribution for both groups are given in detail in Tables 1 and 2.

Our follow-up method as described earlier[3] is: In each case, before therapy was started, several basal metabolic rate (BMR) determinations were obtained. During the first two weeks of therapy, BMR determinations were made twice a week and thereafter at weekly intervals. Determinations of the protein bound iodine (PBI) in the plasma were obtained before therapy commenced. Follow-up determinations were rendered unsatisfactory[2,3,11] by the high iodine content of the two compounds used.

Tracer uptake studies with radioactive iodine were performed before treatment in almost all the cases, and re-tracer studies during the course of treatment were made as follows:

Group I includes patients that were controlled with the medication used and are considered euthyroid. A re-tracer is given from 15 to 24 hours after the discontinuing of medication and a 24-hour uptake over the thyroid gland is taken.

Group II includes patients that were not controlled by the drug used as treatment and who, at the time of re-tracer, showed signs and symptoms of hyperthyroidism. A re-tracer is given from 15 to 48 hours after the discontinuing of medication, and a 24-hour uptake over the thyroid gland is taken.

Of 59 patients treated with Itrumil, 8 failed to respond. Six of these 8 patients are included in this group. Of 11 patients treated with thiouracil plus potassium iodide, one failed to respond and is included in this group.

Microhistometric determinations by the method of Starr and Rawson[13] were made on the glands removed at operation from 12 patients treated with Itrumil and from 5 patients

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TABLE 1.—MAXIMAL CLINICAL RESPONSE IN PATIENTS TREATED WITH ITRUMIL

				Initial				Maximal Clinical Response		
Patient	Age	Sex	Dose of Iodo-Thiouracil	I^{131} Uptake %	PBI μg./100 cc.	BMR %	Pulse	BMR %	Pulse	Days Required
F. G.	20	M	200 mg.	44	17.6	+51	100	+ 6	72	7
G. G.	24	F	200	—	18.8	+46	112	− 3	88	7
K. A.	36	F	600	—		+53	132	− 4	88	7
K. M.	48	F	200	49	17.7	+89	fibrillating	−20	80	7
H. E.	74	F	200	58	11.1	+24	fibrillating	−12	fibrillating 72	
B. D.	43	F	200	—	11.1	+36	92	+ 6	68	8
M. P.	29	F	600	90	15.8	+38	100	− 3	72	8
G. E.	48	F	400	92	16.4	+31	fibrillating	− 3	64	8
B. C.	16	F	400	66	—	+23	96	+ 3	72	9
P. V.	46	F	200	—	10.7	—	112	−14	60	9
E. O.	54	F	200	75	16	+42	88	−11	64	10
B. M. A.	49	F	200	66	14.2	+28	100	+ 6	80	11
C. H.	35	F	200	64	10.3	+37	88	+ 4	76	11
R. M.	43	F	400	92	14.8	—	120	− 8	88	11
D. A.	7	F	300	76	15.2	—	100	clinically controlled		12
E. R.	14	M	200	75	10.1	+15	110	0	74	14
H. E.	76	F	200	65	10.7	+42	fibrillating	− 3	fibrillating	14
T. V.	21	F	300	72	10.8	+16	140	0	80	14
H. A.	25	F	400	94	16.6	+46	80	0	68	14
G. L.	39	F	600	54	13.9	+25	112	—	82	15
D. A.	7	F	200	95	—	—	135	clinically controlled		15
T. B.	17	F	400	75.6	—	+40	120	+ 3	84	15
W. G.	46	F	600	60	11.9	+53	100	+14	78	16
E. R.	14	M	200	65	18.9	+36	124	+ 7	80	17
S. V.	44	F	200	45	15.8	+58	108	+14	80	18
F. Z.	43	F	400	—	21.0	+39	90	− 8	72	18
G. B.	50	F	400	62	—	+23	96	−18	86	19
M. B.	31	F	400	—	23.1	—	112	—	84	19
H. C. H.	39	M	600	41	18	+54	116	+ 4	72	19
P. C.	29	F	400	78	17.1	+43	88	+16	78	21
M. O.	42	F	200	—	16.4	+31	112	+ 8	84	21
W. J.	53	F	600	67	10.5	+59	112	+10	105	22
N. M.	41	F	200	77	14.7	+25	82	+ 8	70	22
D. L.	55	F	400–200	100	—	+35	90	+14	74	23

TABLE 2.—MAXIMAL CLINICAL RESPONSE IN PATIENTS TREATED WITH THIOURACIL PLUS POTASSIUM IODIDE

				Initial				Maximal Clinical Response		
Patient	Age	Sex	(Dose = Itrumil) Thiouracil + KI	I^{131} Uptake %	PBI μg./100 cc.	BMR %	Pulse	BMR %	Pulse	Days Required
G. P.	20	F	400 mg.	57	—	+22	88	+ 3	76	16
A. L.	44	F	400	73.2	16.5	—	120	+11	70	16
K. E.	55	F	800–400	73	12.5	+20	94	−23	84	18
H. T.	30	F	400	86	7.9	+12	96	−12	64	23
Y. L.	47	M	400	63.5	13.8	+43	84	+ 6	60	26
D. L.	38	F	400	63	14	+51	98	+ 6	82	38
W. M.	18	F	400	72	13.7	+34	108	+ 8	78	45
T. O.	37	F	400	92 62	9.8	+18	92	− 4	76	45
R. R.	27	F	400	87	19.8	+46	110	+ 5	80	52
Y. B.	27	F	400	89	20	+71	104	+ 3	56	58

TABLE 3.—COMPARISON BETWEEN THE CLINICAL RESPONSE OF THE ITRUMIL AND THE THIOURACIL PLUS POTASSIUM IODIDE PATIENTS

Number of Patients	PBI μg./100 cc.	I^{131} Uptake %	BMR %	Days to Control	BMR %	Pulse	Dose Range
34	15.3	70.2	+39	13.76±5.5 7–23	+1	77	Iodothiouracil Itrumil 200–600 mg.
10	14.2	74	+35	33.7±15.8 16–58 p = <.001	0	73	Thiouracil + KI 400–800 mg.

treated with thiouracil plus potassium iodide. Part of these findings have been reported earlier[4].

Results. The degree of anti-thyroid action was studied by maximal clinical response and the blocking of the radioactive iodine uptake.

The maximal clinical response, as measured by the decrease in basal metabolic rate and pulse rate, the gain in weight and the disappearance of the symptoms of hyperthyroidism, was obtained in 34 patients treated with Itrumil in an average of 13.76±5.5 days with a range of 7 to 23 days and the dose of 200 to 600 mg. per day. Detailed description is given in Tables 1 and 3.

In 10 cases treated with thiouracil plus potassium iodide with a daily dose of 400 to 800 mg., the maximal clinical response was obtained in an average of 33.7±15.8 days, with a range of 16 to 58 days (Tables 2 and 3).

Comparison of BMR after maximal clinical response has brought out another difference:

Figures 1 and 2 show basal metabolic rate curves of 10 patients with rapid clinical remission who were observed on medication after maximal response. Five patients (Fig. 1) received treatment with Itrumil in dosage ranging from 50 to 600 mg. per day for a period of more than 6 weeks. Five patients (Fig. 2) received treatment with thiouracil plus potassium iodide with a dose of 400 mg. per day for a period of more than 6 weeks. Comparing the tracing of these two groups with the curves (Fig. 3) published by Starr *et al.*[14] on patients that responded with rapid clinical remission with treatment of Lugol's solution, we note:

1). That the Itrumil curves descend quite rapidly (1 to 3 weeks) to zero levels, and are sustained afterwards in the zero or minus range.

2). That the thiouracil plus potassium iodide curves show a slow but consistent decrease in the basal metabolic rate and the zero level is reached after 4 to 8 weeks of therapy remaining in the minus or zero level during the time of observation.

3). That the curves after treatment with Lugol's solution show the clinical iodine effect with lowering of the basal metabolic rate to normal plus levels. These curves, however, return after 3 weeks of treatment to the original pathological increased values.

This comparison suggests that the Itrumil group responds differently from the thiouracil plus potassium iodide and Lugol's solution groups. A greater number of patients will have to be studied in order to establish a definite conclusion.

Radioactive Iodine Uptake. In Group I, 11 patients, who were fully controlled with the medication administered and in the euthyroid state, were studied. Of these 11 patients, 8 were treated with Itrumil with a dose range of 50 to 600 mg. per day, for a period of time varying from 20 to 365 days. Their pulse average was 78, and BMR average was —4. The drug was discontinued from 15 to 24 hours prior to the administration of the tracer dose and the 24-hour uptake of the thyroid gland gave an average of 13.2%, with a range of 5 to 21% (Table 4 and Fig. 4). Their average pretreatment radio-iodine uptake was 70%.

Three patients were treated with thiouracil plus potassium iodide in a daily dose of 200 to 400 mg. for a period of 56 to 78 days. After the euthyroid state was reached (pulse average of 68 and BMR average of —8), the drug was discontinued 24 to 36 hours prior to the administration of the tracer dose and the 24-hour uptake of the thyroid gland gave an average of 21.7% with a range of 10 to 35% (Table 4 and Fig. 4). Their uptake before treatment averaged 82%.

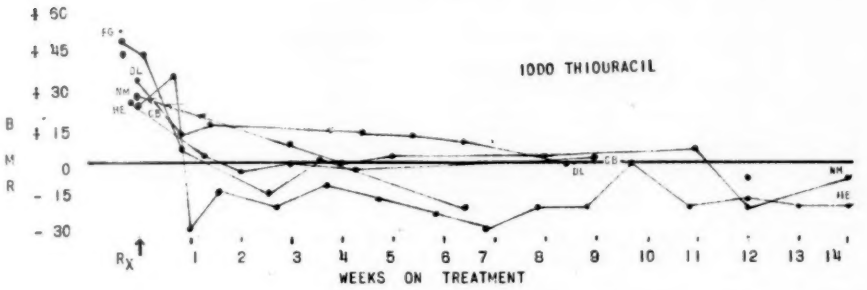

FIG. 1.—Basal Metabolic Rate curves of 5 patients that received treatment with Itrumil for a period of more than 6 weeks and had a rapid clinical remission.

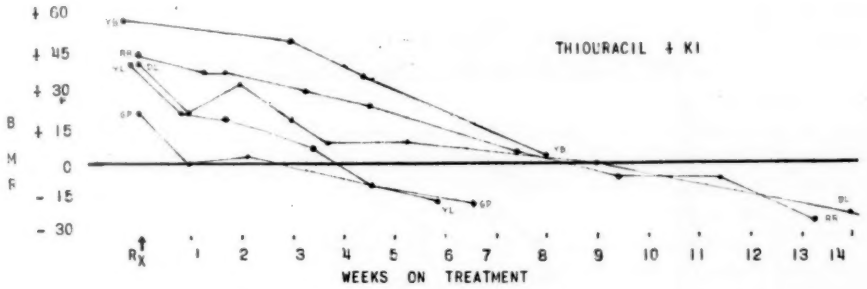

FIG. 2.—Basal Metabolic Rate curves of 5 patients that received treatment with thiouracil plus potassium iodide in amounts equivalent to doses of Itrumil for a period of more than 6 weeks and had a rapid clinical remission.

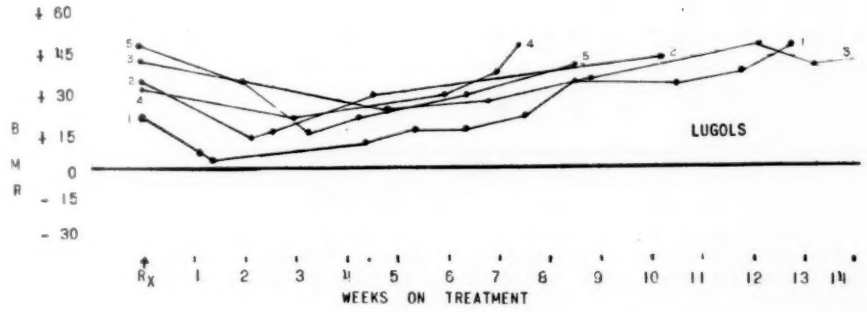

FIG. 3.—Basal Metabolic Rate curves of patients treated with Lugol's solution with a rapid clinical remission. Taken from Starr *et al.*, Arch. Int. Med., **34**, 355, 1924.

TABLE 4.—DETAILED VALUES OF THE 24-HOUR THYROID UP-TAKE OF THE CONTROLLED PATIENTS AFTER HAVING DISCONTINUED EITHER ITRUMIL OR THIOURACIL PLUS POTASSIUM IODIDE FOR A PERIOD OF 15 TO 36 HOURS PRIOR TO THE RETRACER

Patient	*Initial I^{131} Uptake* %	*Dose of Itrumil (Iodothiouracil)* mg.	*Duration*	*Period Between Cessation of R and Retracer* hours		*Retracer I^{131} Uptake* %	*Clinical Status*
E. O.	75	200–400	20 Days	15		10	Euthyroid
W. J.	67	600	50 "	15		13	"
G. G.	—	400	60 "	15		5	"
H. E. 1	58	200–50	7 Months	15		5.6	"
H. E. 2	65	200–100	78 Days	24		14.8	"
D. A.	76	300	21 "	24		21	"
N. M.	77	200–50	12 Months	18		19	"
P. C.	71	400–50	148 Days	17		17	"
8 Cases					Average	13.2%	
		Thiouracil + KI (Dose = Itrumil) mg					
H. T.	86	200–400	78 Days	24		35	Euthyroid
Y. B.	89	400	56 "	24		10	"
W. M.	72	400	69 "	36		20	"
3 Cases					Average	21.7%	

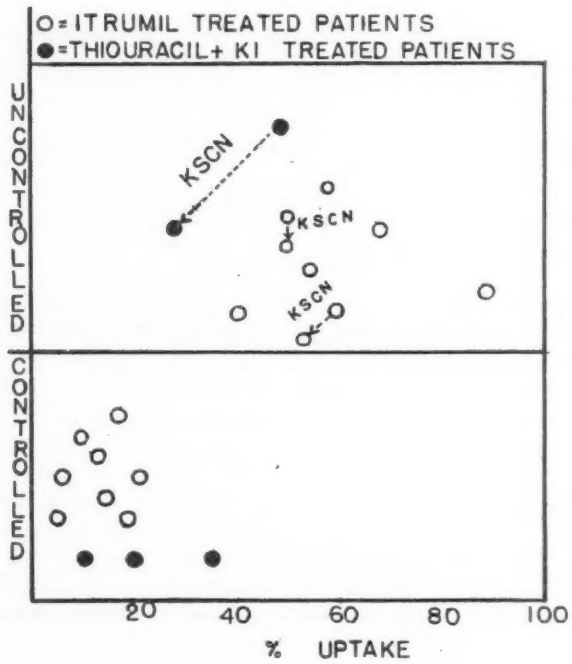

FIG. 4.—Twenty-four hour thyroid up-take on patients that were receiving either Itrumil or thiouracil plus potassium iodide and the drug was discontinued from 15 to 48 hours prior to tracer dose.

The upper part of the figure shows the results on the patients that were not controlled by the medication.

The lower part of the figure shows the patients that were considered euthyroid at the time of the re-tracer.

A greater number of patients are being studied in our clinic to establish the significance of these differences between the Itrumil and thiouracil plus potassium iodide groups.

In Group II, 7 patients that remained hyperthyroid during the course of treatment were studied. Six of them received treatment with Itrumil with a daily dose of 200 to 600 mg. for a period of 43 to 155 days. The pulse average was 106. Their pre-treatment uptake average was 74%. After the drug was discontinued for 24 to 48 hours, a re-tracer dose was administered and the 24-hour uptake over the thyroid gland revealed an average uptake of 60.3%, with a range of 39.7 to 83%. As seen in Table 5 and Fig. 4, patient E. R., who had a second re-tracer after a second course of therapy, showed a 24-hour uptake of 50% and 1 hour after 1 gm. of potassium sulfocyanide was given the uptake remained unchanged.

Case P. E. showed an uptake of 59% and 1 hour after 1 gm. of potassium sulfocyanide the uptake was 53%.

One patient who received thiouracil plus potassium iodide as treatment was available for this study. As seen in Table 5 and Fig. 4, Case M. B. remained hyperthyroid after 36 days on 400 mg. of thiouracil plus potassium iodide. The pulse rate average was 110 and the pre-treatment uptake was 60%. The drug was discontinued for 15 hours prior to the re-tracer dose. A 24-hour uptake over the thyroid gland revealed 49% and 1 hour after potassium sulfocyanide the uptake was 28%.

The degree of goitrogenic action was measured by clinical estimation of the change in thyroid size and by the microhistometric studies with determination of the mean acinar cell height (MACH).

The changes in thyroid size were measured by clinical observation and cannot be considered as an accurate method.

No increase in the thyroid size was observed in the 34 cases treated with Itrumil (Table 6).

Of the 10 cases treated with thiouracil plus potassium iodide, 6 showed a definite enlargement of the thyroid gland during the course of therapy, and in 3, transient tenderness over the thyroid gland was observed during the first 10 days of treatment (Table 6).

The mean acinar cell height determinations were performed in 12 glands treated with Itrumil in dosage of 100 to 600 mg. per day for a period of 13 to 64 days, and in 5 glands treated with 400 mg. of thiouracil plus potassium iodide per day for a period of 29 to 91 days (Table 7).

Only cases of non-nodular goiter and thyrotoxicosis confirmed by pathological study are reported here. In all the cases, sections were examined in from 2 to 4 different blocks of the same surgical specimens.

In the Itrumil treated glands, the range of the average MACH of each gland was from 4.9 to 11.7 micra; the total average was 8.5 ± 2.0 micra (Table 7).

In the thiouracil plus potassium iodide treated glands, the range of the average MACH of each gland was from 6.8 to 13.6 micra; the total average was 10.9 ± 2.9 micra (Table 7).

Discussion. The experimental studies done in rats and chicks by Gassner *et al.*[6] and Barrett *et al.*[1] showed a strong anti-thyroid action and almost no goitrogenicity of Itrumil. The aforementioned authors were not able to reproduce these results with an equimolar mixture of thiouracil plus potassium iodide. Our data tends to confirm their findings.

The maximal clinical response in the patients treated with Itrumil is accomplished earlier (13.76 ± 5.5 days)

TABLE 5.—DETAILED VALUES OF THE 24-HOUR THYROID UP-TAKE OF THE UNCONTROLLED PATIENTS THAT RECEIVED EITHER ITRUMIL OR THIOURACIL PLUS POTASSIUM IODIDE HAVING DISCONTINUED THEIR MEDICATION FOR A PERIOD OF 15 TO 48 HOURS PRIOR TO RETRACER

Patient	*Initial I^{131} Uptake* %	*Dose of Itrumil (Iodothiouracil)* mg.	*Duration*	*Period Between Cessation of R and Retracer* hours	*Retracer I^{131} Uptake* %	*Clinical Status*
J. A.	88	200	3 Months	48	54	Hyperthyroid
H. J.	61	400–300	4 "	48	68	"
M. E.	62	300	43 Days	24	58	"
E. R. 1	65	200	78 "	26	83	"
E. R. 2	83	400–200	94 "	24	50% KSCN 50%	"
P. E.	71	400–200–600	155 "	36	59% KSCN 53%	"
J. J.	88	400–200	55 "	24	39.7	"
6 Cases					Average 60.3%	
		Thiouracil + KI (Dose = Itrumil) mg.				
M. B.	60	400	36 Days	15	49% KSCN 28%	Hyperthyroid

TABLE 6.—COMPARISON OF THE GOITROGENICITY OF THIOURACIL PLUS POTASSIUM IODIDE IN CONTRAST WITH THE LACK OF GOITROGENICITY OF ITRUMIL

Dose	*Number of Patients*	*Thyroid Enlargement*
Iodothiouracil 200–600 mg.	34	None
Thiouracil + KI 400–800 mg.	10	6 Transient tenderness in 3 of them

TABLE 7.—MEAN ACINAR CELL HEIGHT DETERMINATIONS (MACH) OF THE GLANDS REMOVED BY SURGERY FROM PATIENTS TREATED WITH EITHER ITRUMIL AND THIOURACIL PLUS POTASSIUM IODIDE

Patient	*M.A.C.H.*	*Pre-operative Status* *BMR %*	*Pulse*	*Dose of Iodothiouracil (Itrumil)*	
F. G.	4.9	+1	68	100–400 mg.—64 Days	
K. M.	6.3	+6	76	600	40
B. M.	7.4	0	72	200	39
G. G.	7.5	+3	78	200–400	62
E. O.	7.7	−17	64	200–400	22
G. E.	8.2	+3	76	400	19
M. P.	8.9	−3	72	600	13
C. H.	9.0	−6	72	600–100	25
K. A.	9.6	+4	96	600	34
G. L.	9.7	—	80	600	28
S. V.	11.6	+6	76	200	40
W. J.	11.7	+10	100	600	60
12 Cases	8.5±2.0 Micra	Average			
				Dose of Thiouracil + KI Dose = Itrumil	
A. L.	6.8	−3	70	400	29
W. M.	9.2	−16	68	400	91
G. P.	11.7	−16	56	400	45
Y. L.	13.3	−17	60	400	49
Y. B.	13.6	+3	56	400	68
5 Cases	10.9±2.9 Micra	Average			

than in those treated with equimolar amounts of thiouracil plus potassium iodide (33.7±15.8 days), and the difference was found to be statistically significant, namely; p of less than 0.001.

Of the 10 patients treated with thiouracil plus potassium iodide, 6 of them showed definite enlargement of their thyroid glands and in 3 of these 6 cases, a transient tenderness over the thyroid during the first 10 days of treatment was observed. This indicates a definite goitrogenic action in contrast with the lack of goitrogenicity observed with the use of Itrumil.

The mean acinar cell height determinations, which in our opinion give a more consistent and accurate analysis of the thyroid structure, showed in the Itrumil treated glands, a lower average of 8.5±2.0 micra than the average of 10.9±2.9 micra observed for the glands treated with thiouracil plus potassium iodide. To establish a statistical significance, a greater number of cases should be analyzed.

The above findings indicate a difference of the mode of action of Itrumil and thiouracil plus potassium iodide. The results of the tracer studies with radioiodine show that the euthyroid group which received the iodinated thiouracil compounds had an average uptake over the thyroid gland of 13% (Itrumil) and 21.7% (thiouracil plus potassium iodide), when the drugs were discontinued 15 to 24 hours prior to the administration of the tracer dose.

The patients that remained hyperthyroid under treatment with Itrumil revealed, when a re-tracer was given from 15 to 48 hours after discontinuing the medication, an average uptake of 65%. Patients E. R. and P. E. (Fig. 4) showed no change in the uptake 1 hour after administering potassium sulfocyanide.

Williams *et al.*[16], Skanse[12], Werner[15], and Freedberg *et al.*[5], have found that the administration of stable iodine as treatment will block the collection of radioiodine for at least 1 to 12 weeks. Our group of uncontrolled patients, in spite of having received from 200 to 600 mg. of iodinated thiouracil compounds for a period of 36 to 55 days, were able to concentrate and bind (iodinate[18]) the radioactive iodine administered as a tracer after discontinuing medication for 24 to 48 hours before the tracer dose was given. Wolff and Chaikoff[18] demonstrated that if the iodine circulating in the plasma is not maintained above 30 micrograms per 100 cc. the accumulation and iodination mechanism in the thyroid gland will be restored. It is possible that Itrumil, which is absorbed and excreted in great part unchanged[10], if not taken up by the hyperthyroid gland, will be rapidly eliminated with consequent lowering of the circulating plasma iodine and the accumulation and iodinating mechanism of the thyroid gland will be unaffected. If, however, the Itrumil produces a therapeutic response, the level of circulating Itrumil is maintained elevated and the trapping mechanism of the thyroid is blocked.

The different mode of action of Itrumil compared with the equivalent molecular quantities of thiouracil plus potassium iodide, can be hypothetically explained by assuming that the rapid control of thyrotoxic symptoms accomplished with Itrumil is due to an effective and selective concentration of this iodinated molecule in the hyperthyroid gland. We may assume that once this iodinated thiouracil is absorbed into the cellular structure of the thyroid gland, an inhibition of thyroxin hormone takes place in the cell itself.

It can also be assumed that Itrumil simultaneously raises the iodine concentration and inhibits the production or release of the thyrotropic stimulating hormone (TSH) from the pituitary. Once the thyrotropic stimulating hor-

mone production and the local action of TSH in the thyroid cell is inhibited or decreased, the thyroid gland has an opportunity to be restored to normal histology and normal physiological conditions.

The selected cases illustrated in Figs. 1, 2, and 3 indicate a striking difference of action in terms of basal metabolic rate, of iodide alone (Lugol's solution), thiouracil and iodide, and Itrumil. The basal metabolic rate of the patients treated with Itrumil responded even more rapidly than the Lugol's solution group. In these Itrumil cases, the basal metabolism levels were maintained near the zero level as long as observation was continued. This indicates further differences in clinical action of the synthetic iodide thiouracil as compared with the other combinations. Only patients that responded with a rapid clinical remission were selected for this comparison. Larger groups will undoubtedly show much greater variations and no statistical conclusion is warranted from the limited data.

Summary. The rapid maximal clinical response (13.76 days average) and lack of goitrogenicity observed in 34 patients treated with Itrumil (Iodothiouracil) was not reproducible with equivalent quantities of thiouracil plus potassium iodide given to 10 hyperthyroid patients.

The mean acinar cell height (MACH) was lower (8.5 micra) in the Itrumil treated gland than in the glands treated with thiouracil plus potassium iodide (10.9 micra).

Radioiodine re-tracers done in both groups after discontinuance of either drug for 15 to 48 hours showed that when the hyperthyroidism is controlled, the 24-hour thyroid uptake is low (13% in the Itrumil treated cases and 21% in the thiouracil plus potassium iodide treated group). If the patient has not responded to treatment his uptake is in the hyperthyroid levels: 65% average.

A specific mode of action of Itrumil is discussed.

The response obtained with Itrumil differs from that obtained with thiouracil plus potassium iodide or Lugol's solution alone. Comparison of the BMR tracings after maximal clinical response in a selected small number of cases treated with these drugs showed that the Itrumil treated patients reached the zero level earlier than the others and the basal metabolic rate was maintained near the zero level as long as observation was continued.

REFERENCES

1. **Barrett, H. W., Gassner, F. X.,** and **Dittmer, K.:** Endocrinology, **48**, 189, 1951.
2. **Bondy, P. K.:** J. Clin. Endocrinol., **11**, 1406, 1951.
3. **Catz, B., Petit, D. W.,** and **Starr, P.:** Ibid., **11**, 978, 1951.
4. **Catz, B.,** and **Starr, P.:** Ibid., **12**, 228, 1952.
5. **Freedberg, A. S., Chamovitz, D. L.,** and **Kurland, G. S.:** Metabolism: Clin. and Exp., **1**, 36, 1952.
6. **Gassner, F. X., Hopwood, M. L., Herrold, E. A.,** and **Plummer, A. J.:** J. Clin. Endocrinol., **10**, 1485, 1950.
7. **Goldner, M. G.,** and **Kirschenfeld, S.:** Metabolism: Clin. and Exp., **1**, 111, 1952.
8. **McClintock, J. C.,** and **Lyons, J. A.:** J. Clin. Endocrinol., **11**, 111, 1951.
9. Iidem: New York J. Med., **51**, 1633, 1951.
10. **Plummer, A. J.:** Fed. Proc., **9**, 309, 1950.
11. **Rapport, R. L.,** and **Curtis, G. M.:** J. Clin. Endocrinol., **10**, 735, 1950.
12. **Skanse, B.:** Acta med. scandinav., (supp. 235), **136**, 1, 1949.
13. **Starr, P.,** and **Rawson, R. W.:** Proc. Soc. Exper. Biol. & Med., **35**, 603, 1937.
14. **Starr, P., Walcott, H. P., Segall, H. N.,** and **Means, J. H.:** Arch. Int. Med., **34**, 355, 1924.
15. **Werner, S., Hamilton, H., Leifer, E.,** and **Goodwin, L.:** Clin. Endocrinol., **9**, 1054, 1949.
16. **Williams, R., Jaffe, H.,** and **Taylor, J.:** Am. J. Med. Sci., **219**, 7, 1950.
17. **Williams, R. H., Towery, B. T., Rogers, W. F., Jr., Tagnon, R.,** and **Jaffe, H.:** J. Clin. Endocrinol., **9**, 801, 1949.
18. **Wolff, J.,** and **Chaikoff, I. L.:** Endocrinology, **42**, 468, 1948.

PHOSPHATASE ACTIVITY AS A GAUGE OF ACID INFLUENCE IN PEPTIC MUCOSAL DAMAGE*

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Most authorities agree that the importance of gastric acid secretion in the perpetuation of gastro-duodenal ulceration is established. What remains in doubt, and the subject of much controversy and experiment, is the part this secretion plays in the actual initiation of these lesions; how much the necrosis of the erosion and acute ulcer owes to its corrosive and proteolytic effects. There are many workers who have found cause to mistrust the role it supposedly plays in this regard, and some who indicate that the prominence given its action in all aspects of peptic ulceration has been overemphasized[6,14,13]. Ivy *et al.*[12] comment that "proof is still lacking that gastric hypersecretion alone initiates the ulcerative process . . . It appears that . . . some other factor or factors operate to impair the vitality of the mucosal cells." On the other hand, to Bolton[3,4] the gastric acid seemed all-important in the causation of mucosal defects and without its action necrosis of cells would not occur. Thus he writes[3], "the actual necrosis of cells, as seen under the microscope, is brought about by the action of the gastric juice acting upon a cell in some way changed, but showing no microscopic deviation from normal."

During recent work by one of us (J.H.F.) upon tissues from examples of human gastric and duodenal erosions and pre-erosive necrotic lesions, it was necessary to question whether or not the necrosis and the cytological changes observed within the mucosal cells deeper than the floor of the erosions were due to the penetration of gastric acid to this depth. In the experiments of Light *et al.*[15] the chromatic change of the hemoglobin within the erythrocytes of concomitant erosion hemorrhages was utilized in an attempt to decide this question. They write, "The change of color which accompanies the breakdown of the erythrocytes is an indication of the depth to which the acid chyme has penetrated into the tissues." While it is admitted that this process of assessment may have some degree of reliability, it would be an advantage to have an additional method whereby the involvement of secreted acid in peptic mucosal necrobiosis may be supported or denied.

Several cellular components of the gastric and duodenal mucosa have a particularly rich content of alkaline phosphomonoesterase[5,10,11,16] which can be simply and consistently demonstrated by Gomori's[9,10] histochemical technique. Moreover, this substance be-

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comes extremely concentrated in the vicinity of erosive and ulcerative damage[8]. It was supposed that an enzyme requiring distinctly alkaline conditions for maximum action on its substrate might serve as a tool in estimating the depth of penetration of acid into the tissue. The acid, free in the tissues, might irreversibly inactivate this enzyme, and if so, the depths to which gastric juice penetrates the walls of the stomach and duodenum in peptic mucosal necrosis might be determined. Contrary to expectations, references to the effects of acid and gastric juice upon the enzyme could not be found. Thus, before comment could be made upon the possible usefulness of this idea it was necessary to assay the activity of the enzyme and its survival after incubation in media of acid and gastric juice. Accordingly a preparation of crystalline intestinal alkaline phosphatase was procured* and experiments were conducted to elucidate this question.

Method. Phosphatase assays were carried out using a sodium betaglycerophosphate substrate[1,2] with the addition of 0.01 M magnesium ion. Phosphorus analyses were performed according to the method of Fisk and Subbarrow[7]. The crystalline alkaline phosphatase was dissolved in either water, acid, or gastric juice, at a concentration of 0.1 mg. per cc. The solution was then incubated for 1 hour at 37° C. with continuous slow agitation. Then 0.1 cc. of the incubated solution was transferred to 8 cc. of the glycerophosphate substrate and incubated under the same conditions for 30 minutes. Phosphate analyses were then carried out. Blank determinations were performed by the transfer of 0.1 cc. of water, acid, or gastric juice to the substrate without the addition of enzyme.

TABLE 1.—RESULTS OF PHOSPHATE ANALYSES AFTER PRE-TREATMENT OF PHOSPHATASE ENZYME WITH WATER, HYDROCHLORIC ACID SOLUTIONS, AND GASTRIC JUICE FROM NORMALS AND PEPTIC ULCER PATIENTS

Pre-Treatment of Enzyme		*Micrograms of Phosphorus Released After Treatment*	*Remarks*
Solvent	*pH*		
Water	—	29	Average of 4 determinations
Hydrochloric Acid solutions	2.4	0	2 determinations
" " "	3.2	0	4 determinations
" " "	4.2	31	Average of 2 determinations
Gastric Juice	1.83	0	Patient A. (D.U.)*
" "	2.2	0	Patient B. (G.U)**
" "	1.8	0	Patient C. (D.U)
" "	1.65	0	Patient D. (D.U)
" "	8.0	41	Patient E. (Nil)
" "	6.9	41	Patient F. (G.U)
" "	7.65	26	Patient G. (Nil)
" "	7.4	30	Patient H. (Nil)

* D.U. = Duodenal Ulcer ** G.U. = Gastric Ulcer

NOTE: As described in the text the enzyme was first incubated in the solvents listed and then its activity to the glycerophosphate substrate determined. The gastric juice was obtained in the course of routine gastric analyses on patients clinically suspected of peptic ulceration. (Courtesy of the Victoria General Hospital, Halifax.)

Results. The results of these experiments are presented in Table 1.

Conclusions. These experiments indicate that alkaline phosphatase is inactivated by exposure to acid concentrations lower than pH4 for 1 hour at

* Delta Chemical Works, 23 West 60th Street, New York 23, New York.

37° C. While this inactivation may not be absolutely irreversible, there is no step in the histochemical unmasking of the enzyme likely to reactivate it, should it have been exposed to such hydrogen ion concentrations for any appreciable length of time. In addition, there appears to be no significant protective effect of gastric juice on alkaline phosphatase so exposed. Although it remains possible that the enzyme may behave differently in the cell than when extracted, the authors feel that, provisionally, the presence of the pigmented precipitate in a tissue section may be taken to mean that the catalytic agent responsible for its origin has not been subjected for any length of time to pH values below 4. This is considered to be more significant when such a precipitate is seen within degenerate cells and amidst necrotic debris. Accordingly it is believed that this staining may have considerable value in determining the depth of penetration of acid gastric juice in mucosal denudative and necrotic foci.

Summary. It has been shown that alkaline phosphomonoesterase of intestinal origin is inactivated by 1 hour exposure at 37° C. to acid concentrations below pH4. In addition, no protective effect of gastric juice on the enzyme can be determined. It is, therefore, suggested that the histochemical stain for phosphatase may be utilized as a guide to the depths of penetration of acid at the sites of peptic mucosal damage.

REFERENCES

1. **Bodanski, A.**: J. Biol. Chem., **99**, 197, 1932.
2. Idem: Ibid., **101**, 93, 1933.
3. **Bolton, C.**: J. Path. & Bact., **14**, 418, 1910.
4. Idem: Ibid., **20**, 133, 1915.
5. **Bourne, G.**: Quart. J. Exp. Physiol., **32**, 1, 1943.
6. **Faber, K.**: Gastritis and its Consequences, Oxford Medical Publications, London, p. 21, 1935.
7. **Fisk, C. H.**, and **Subbarrow, Y.**: J. Biol. Chem., **66**, 375, 1925.
8. **Fodden, J. H.**: To be published.
9. **Gomori, G.**: Proc. Soc. Exp. Biol. & Med., **42**, 23, 1939.
10. Idem: J. Cell. and Comp. Physiol., **17**, 71, 1941.
11. Idem: Am. J. Clin. Path., **16**, 347, 1946.
12. **Ivy, A. C., Grossman, M. I.**, and **Bachrach, W. H.**: Peptic Ulcer, The Blakiston Company, Philadelphia, p. 224, 1950.
13. **James, A. H.**, and **Pickering, G. W.**: Clinical Science, **8**, 181, 1949.
14. **LeVeen, H. H.**: Gastroenterology, **8**, 648, 1947.
15. **Light, R. U., Bishop, C. C.**, and **Kendall, L. G.**: J. Pharmacol. & Exp. Therap., **45**, 227, 1932.
16. **Newman, W., Feigin, I., Wolf, A.**, and **Kabat, E. A.**: Am. J. Path., **26**, 257, 1950.

BENZETHACIL (BICILLIN)*: A REPORT OF THE PENICILLEMIA FOLLOWING ITS ORAL ADMINISTRATION IN MAN

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THERE is increasing evidence of the therapeutic effectiveness of penicillin administered by oral route in the management of disease caused by penicillin-sensitive organisms[1,2,11,14,16,17,19]. The development of benzethacil (N,N[1] Dibenzylethylenediamine Dipenicillin), which is nearly insoluble in water[23] (approximately 200 units/cc. at 40°C.) makes possible the preparation of stable and palatable suspensions for oral administration. This has been impractical heretofore by reason of the disagreeable taste of soluble penicillin salts and their instability in solution. Since other insoluble penicillin salts previously studied[3,4,5] have failed to demonstrate any superiority over potassium penicillin in terms of the penicillemia resulting from their administration, it seemed purposeful to study benzethacil in this regard. Accordingly, we have studied the penicillemia resulting from benzethacil suspension given as a), a single oral dose of 300,000 units; b), the fourth 300,000-unit dose given every 6 hours; c), a single oral "loading" dose of 600,000 units; and d), a single oral dose of 300,000 units of benzethacil when compared in the same patients with an equal dose of potassium penicillin G.

It was hoped that the data so obtained might enable us to predicate a schedule of therapy that could be reliably employed for the treatment of infections due to organisms readily susceptible to penicillin and to compare this, as regards penicillemia, with currently employed schedules of therapy using orally administered potassium penicillin G. Previous work has demonstrated enhancement by probenecid** of the penicillemia in man following a given dose of any form of penicillin thus far tested[6,7,8,13,18,21,22,24,25,26,27] and it seemed appropriate to determine whether benzethacil behaved similarly.

Methods. Healthy ambulatory subjects with normal renal function as determined by routine urinalysis and BUN determinations, were used in all the studies herein reported.

* Bicillin is the trademark for N,N[1] Dibenzylethylenediamine Dipenicillin. The generic name of this compound is benzethacil. Supplies of benzethacil suspension used throughout this study were made available by Wyeth, Inc., Philadelphia, Pa.

** Probenecid is the generic name for p(di-n-propylsulfamyl) benzoic acid. This preparation is marketed by Sharp and Dohme, Inc. under the trademark Benemid.

All specimens for penicillin assay were submitted to the laboratory with coded designations, the code being employed for correlation of data after the studies were completed.

In STUDY I, 6 individuals with an average age of 27 years (range 20 to 44) and an average weight of 142 pounds (range 118 to 165) were given a single 300,000-unit dose of benzethacil orally, alone and in conjunction with 2 gm. of probenecid in a "crossover" pattern of study. The 5-cc. doses were measured with a syringe and then transferred to medicine glasses to insure accuracy for the purposes of this comparison. Heparinized blood specimens drawn at 2, 4, 6, and 8 hours after administration were centrifuged and separated within one-half hour after collection, then held at −10°C. until submission for the estimation of the penicillin concentration in the plasma. The penicillin assays were done by the *Sarcina lutea* cup-plate method[12], using normal human plasma to dilute the standard.

In STUDY II, the same 6 individuals with an average age of 46 years (range 26 to 56) and an average weight of 161 pounds (range 132 to 214) were used as their own controls in a "crossover" design. On 6/24/52 three patients received at 8 a.m. a dose of 600,000 units of benzethacil orally, followed by 300,000 units given every 6 hours for 4 doses. On the same day, 3 other patients received the same dose of benzethacil as just described, and in addition 1 gm. of probenecid at 8 a.m., followed by 0.5 gm. every 6 hours for 4 doses. On 7/1/52 the two dosage schedules described were reversed and then administered to the same two groups of 3 men. Blood samples were drawn in both phases of the study at ¼, 2, 4, and 6 hours after the initial dose of 600,000 units and at the same time intervals following the fourth dose of 300,000 units. Specimens were handled as previously described and submitted for penicillin assay.

In STUDY III, 3 penicillin preparations, benzethacil, potassium penicillin G,* and potassium penicillin G plus probenecid* were compared in the same 6 patients whose average age was 34 years (range 23 to 49) and whose average weight was 148 pounds (range 113 to 178.) In an attempt to negate any influence associated with time on the comparison of these three treatments a single oral dose of 300,000 units of each preparation was exhibited to the patients as indicated in Table 1.

Blood specimens were drawn at 1, 2, 4, and 6 hours after the dose and handled in the manner described above.

TABLE 1.—DISTRIBUTION OF PATIENTS AMONG TREATMENTS IN STUDY III

Date	*Benzethacil*		*Pot. penicillin G*		*Pot. penicillin G & probenecid*	
8/1/52	M.H.	C.A.	E.M.	M.M.	M.C.	W.B.
8/4/52	E.M.	M.M.	M.C.	W.B.	M.H.	C.A.
8/7/52	M.C.	W.B.	M.H.	C.A.	E.M.	M.M.

Results. The results of STUDY I have been presented in graphic form in Fig. 1. Benzethacil suspension alone is observed to give adequate penicillemia for 4 hours, but not at 6 or 8 hours. The enhancement of penicillemia by probenecid is observed to be 2 to 4 fold.

Figure 2 presents the average values observed in STUDY II following the 600,000-unit "loading" dose of oral benzethacil with and without probencid. The enhancing effect of probenecid is readily apparent being 1.7 to 3.5 fold during the falling portion of the penicillemia curve. In Fig. 3, the enhancement by probenecid of the penicillemia following the fourth 6-hourly dose of 300,000 units of benzethacil is seen to range from 2.7 to 4.7 fold on the falling portion of the curve. All values in the probenecid phase were 0.03 unit/cc. or above. With benzethacil alone at the 6-hour period, 1 of 6 patients failed to achieve 0.03 unit/cc. after the 600,000-unit dose, and 3 of 6 patients after the fourth dose of 300,000 units. The aver-

* Penalev tablets containing 100,000 units of potassium penicillin G and Remanden tablets containing 0.25 gm. of Benemid and 100,000 units of potassium penicillin G were generously supplied by Sharp and Dohme, Inc., West Point, Pennsylvania.

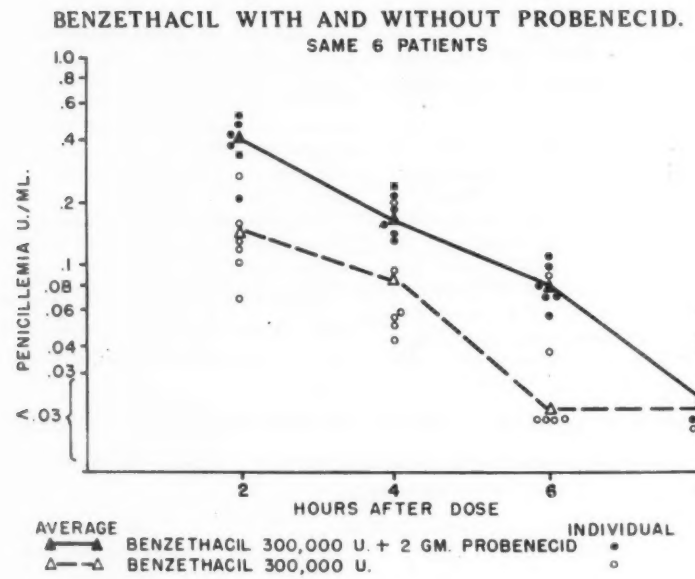
BENZETHACIL WITH AND WITHOUT PROBENECID.
SAME 6 PATIENTS
PENICILLEMIA U./ML.
1.0
.8
.6
.4
.2
.1
.08
.06
.04
.03
< .03
2
4
6
8
HOURS AFTER DOSE
AVERAGE
INDIVIDUAL
BENZETHACIL 300,000 U. + 2 GM. PROBENECID
BENZETHACIL 300,000 U.

FIG. 1

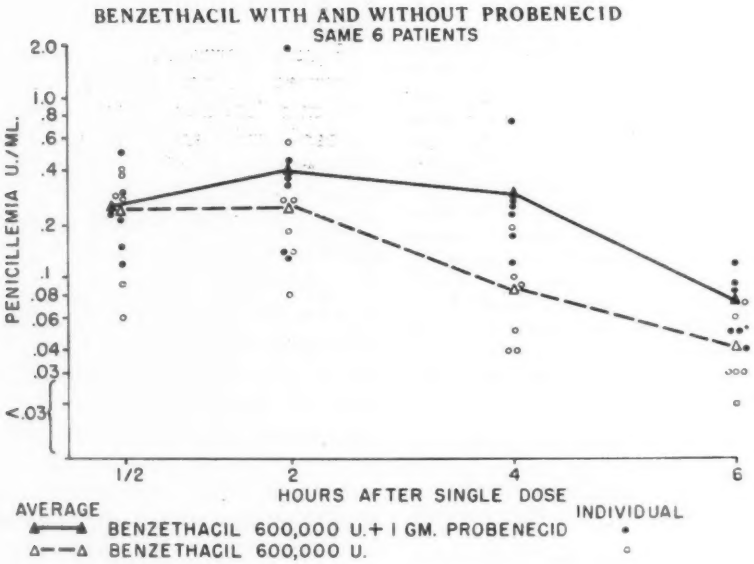
BENZETHACIL WITH AND WITHOUT PROBENECID
SAME 6 PATIENTS
PENICILLEMIA U./ML.
2.0
1.0
.8
.6
.4
.2
.1
.08
.06
.04
.03
< .03
1/2
2
4
6
HOURS AFTER SINGLE DOSE
AVERAGE
INDIVIDUAL
BENZETHACIL 600,000 U.+ 1 GM. PROBENECID
BENZETHACIL 600,000 U.

FIG. 2

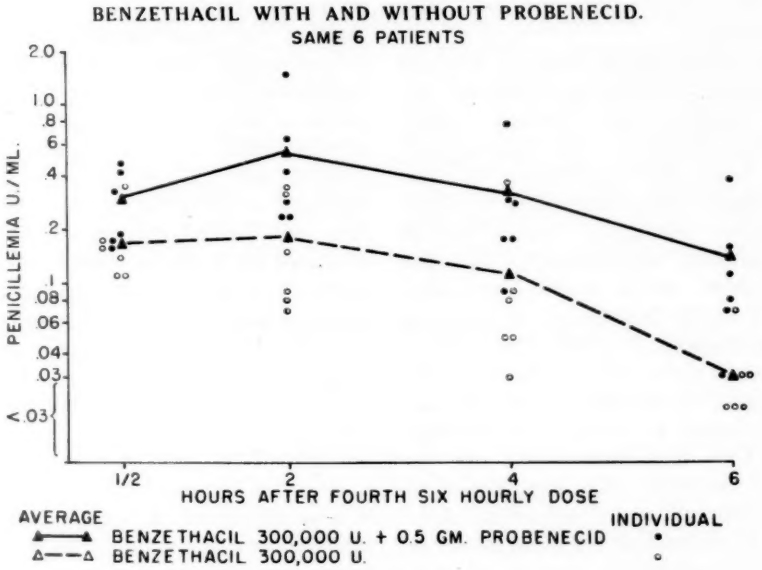
BENZETHACIL WITH AND WITHOUT PROBENECID.
SAME 6 PATIENTS
PENICILLEMIA U./ML.
2.0
1.0
.8
.6
.4
.2
.1
.08
.06
.04
.03
< .03
1/2
2
4
6
HOURS AFTER FOURTH SIX HOURLY DOSE
AVERAGE
INDIVIDUAL
BENZETHACIL 300,000 U. + 0.5 GM. PROBENECID
BENZETHACIL 300,000 U.

FIG. 3

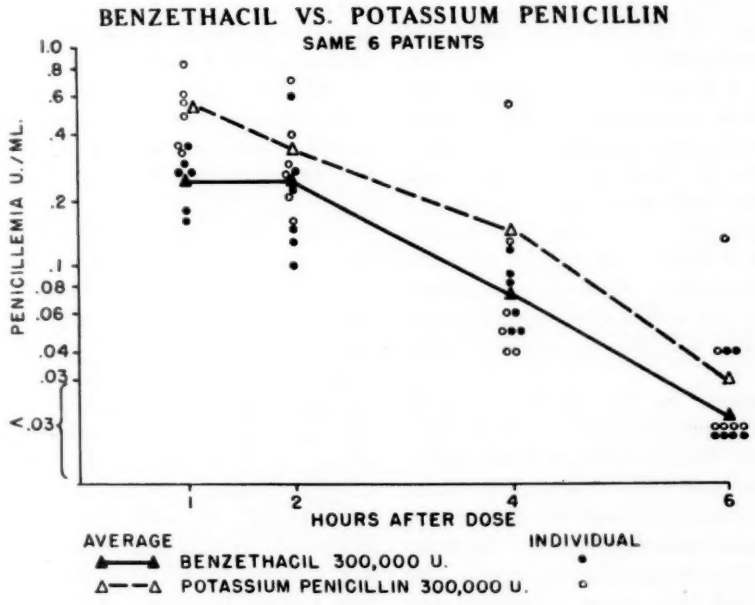
BENZETHACIL VS. POTASSIUM PENICILLIN
SAME 6 PATIENTS
PENICILLEMIA U./ML.
1.0
.8
.6
.4
.2
.1
.08
.06
.04
.03
< .03
1
2
4
6
HOURS AFTER DOSE
AVERAGE
INDIVIDUAL
BENZETHACIL 300,000 U.
POTASSIUM PENICILLIN 300,000 U.

FIG. 4

age values for benzethacil alone, however, were above 0.03 unit/cc. with either dosage.

At the conclusion of STUDY II, 3 values were missing. In order to make the statistical analysis the missing values were estimated by a least squares procedure from the logarithms of the values observed for the rest of the data. While these estimated values cannot be considered replacements for the actual values, which might have been obtained, in the opinion of the authors it is more realistic to graph these values than to omit them when values for the same individual at different periods are to be included in arriving at average values. This has been done. In the statistical analysis the logarithms of the penicillin plasma levels were used in order to normalize the variations observed at the different periods of sampling. The enhancement with probenecid is significant with the greatest effect after the 600,000-unit dose at 4 hours ($P = < 0.01$) and after the fourth dose of 300,000 units at 6 hours ($P =$ about 0.001).

Figure 4 depicts the penicillemia obtained when benzethacil and potassium penicillin G were compared in the same 6 patients. In these patients it is observed that both benzethacil and potassium penicillin G gave satisfactory levels for 4 hours, but not for 6 hours. The comparison between potassium penicillin and potassium penicillin plus probenecid will be presented elsewhere[9].

Discussion. In the earlier days of penicillin therapy when various regimens were being evaluated clinically, the lower limit of the penicillin assay techniques was 0.03 unit/cc. This value has at times been dignified by the term "therapeutically significant level". Depite the fact that some infections can be adequately treated with regimens which attain this level transiently or not at all, and the fact that the assay techniques used in the study will, on occasion, detect levels less than 0.03 unit/cc., we have chosen not to graph values below 0.03 unit/cc. We have represented them arbitrarily as values midway between 0.03 and the baseline of the chart. In the opinion of the authors, few physicians currently recommend a schedule of therapy which does not produce levels in excess of 0.03 unit for a major portion of a given 24-hour period. For the purpose of arriving at average values, levels reported as 0.02 have been valued as such, whereas values reported as less than 0.03 or 0.02 have been considered to be zero.

Figure 5 has been presented for the purpose of summarizing the information pertinent to dosage recommendations for oral benzethacil therapy. Such recommendations would be intended for infections due to penicillin-sensitive organisms which would be expected to respond to a regimen of therapy which maintains a penicillemia of at least 0.03 unit/cc. for most of the 24-hour period. It is observed that the "loading" dose of 600,000 units would seem quite satisfactory by providing prompt therapeutic levels that are equal or slightly superior to those obtained on continuing therapy of 300,000 units given every 6 hours. Although we are aware that it may be treacherous to compare the penicillemia following different doses of penicillin when small numbers of patients are involved and the same patients are not used as their own controls, we have chosen to include in Fig. 5 the average values of 12 patients (6 from STUDY I and 6 from STUDY III) who received a single 300,000-unit dose of benzethacil. It will be noted from reference to the individual values in Fig. 1 and 4 that 8 of these 12 patients had values less than 0.03 unit/cc. at 6 hours.

On the basis of the above information, we would recommend a 600,000-

unit "loading" dose of benzethacil suspension, followed by 300,000 units given every 6 hours thereafter. In other studies[10] contrasting the penicillemia in normal versus ill subjects after a given regimen of injectable penicillin, one of us (W.P.B.) has observed slightly higher levels in the ill group. If factors other than those of absorption from the injection site contribute to this effect, surprisingly similar to those observed after the 3 treatments depicted in Fig. 5. Any further interpretation is difficult in the absence of information on the age, weight, health, or renal functions of the patients previously[15] reported. The pencillemia observed by Lepper and his associates[20] following the administration of a single 600,000-unit oral dose to 5 patients with acute gono-

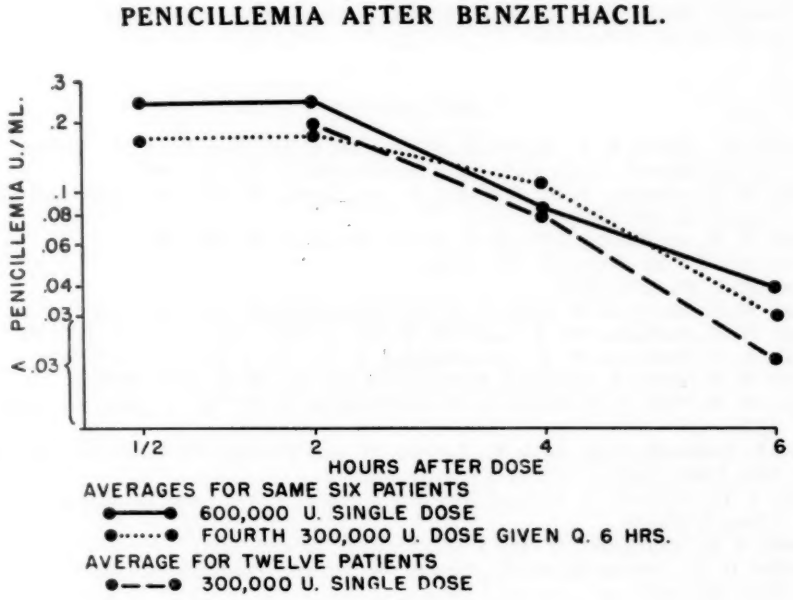

FIG. 5

it may be observed following orally administered benzethacil as well. At the time of this writing, two previous papers have been published which present information on the penicillemia observed after orally administered benzethacil[15,20]. In the paper by Elias *et al.*[15], the average levels of 12 patients after the initial dose of 100,000 units are .099, .119, and .081 unit/cc. at 1, 2, and 4 hours respectively. With the higher doses used in our study, the peak levels are considerably higher. The 4-hour level of .081 unit/cc.[15], however, is coccal urethritis was slightly higher than that observed in our normal subjects.

Summary: A dosage regimen of an initial dose of 600,000 units of benzethacil suspension followed by 300,000 units every 6 hours should be satisfactory for easily treatable infections due to penicillin-sensitive organisms.

Because of the relatively low peak levels obtained, oral penicillin is not recommended for the treatment of infections requiring a high order of peni-

cillemia either to combat a high resistance by the organism or to treat infection in a site to which diffusion of penicillin is poor. Benzethacil is no exception.

Benzethacil orally when given in the same or perhaps a slightly higher dose, may give a penicillemia comparable to that observed after the administration of potassium penicillin G.

Probenecid has been demonstrated to have an enhancing effect of the same magnitude upon the penicillemia following benzethacil administration as that observed with other forms of penicillin.

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REFERENCES

1. **Austrian, R., Mirick, G. S., Rogers, D. E., Sessoms, S. M., Tumulty, P. A., Vickers, W. J., Jr.,** and **Zubrod, C. G.**: Bull. Johns Hopkins Hosp., **88**, 264, 1951.
2. **Boger, W. P., Crosley, A. P., Carfagno, S.,** and **Bayne, G. M.**: Antibiotics and Chemotherapy **2**, 555, 1952.
3. **Boger, W. P.,** and **Beatty, J. O., Jr.**: J. Invest. Dermatol., **17**, 157, 1951.
4. Iidem: Am. J. Clin. Path. **20**, 373, 1950.
5. Iidem: Ibid., **20**, 1070, 1950.
6. **Boger, W. P., Beatty, J. O., Pitts, F. W.,** and **Flippin, H. F.**: Ann. Int. Med., **33**, 18, 1950.
7. **Boger, W. P., Gallagher, M. E.,** and **Pitts, F. W.**: J. Phila. Gen. Hosp., **1**, 51, 1950.
8. **Boger, W. P., Matteucci, W. F.,** and **Schimmel, N. H.**: Am. J. Med., **11**, 517, 1951.
9. **Boger, W. P., Gvlfe, J., Carfagno, S. C.,** and **Bayne, G. M.**: to be published.
10. **Boger, W. P., Oritt, J. E., Israel, H. L.,** and **Flippin, H. F.**: Am. J. Med. Sci., **215**, 250, 1948.
11. **Bunn, P., Caldwell, E. R., Adair, C., Lepper, M.,** and **Dowling, H. F.**: J. Am. Med. Assn., **144**, 1540, 1950.
12. **Burke, J. C.**: Methods in Medical Research, Volume 1, Yearbook Publishing Company, Chicago, Illinois.
13. **Burnell, J. M.,** and **Kirby, W. M.**: J. Clin. Invest., **30**, 697, 1951.
14. **Dowling H. F., Rotman-Kavka, G., Hussey, H. H.,** and **Hirsh, H. L.**: Am. J. Med. Sci., **213**, 413, 1951.
15. **Elias, W., Price, A. H., and Merrion, H. J.**: Antibiotics & Chemotherapy, **1**, 491, 1951.
16. **Finland, M., Meads, M.,** and **Ory, E. M.**: J. Am. Med. Assn., **129**, 315, 1945.
17. **Flippin, H. F., Matteucci, W. V., Schimmel, N. F.,** and **Boger, W. P.**: J. Am. Med. Assn., **147**, 918, 1951.
18. **Flippin, H. F., Matteucci, W. V., Schimmel, N. H., Bartholomew, L. E.,** and **Boger, W. P.**: Antibiotics & Chemotherapy, **2**, 208, 1952.
19. **Hoffman, Wm. S.,** and **Volini, I. F.**: Am. J. Med. Sci., **213**, 520, 1947.
20. **Lepper, M. H., Rodriguez, J., Blatt, N.,** and **Spies, H. W.**: Antibiotics & Chemotherapy, **2**, 175, 1952.
21. **Matis, P., Gross, R., Hebel, R.,** and **Hertel, H.**: Med. Welt, **20**, 1135, 1951.
22. **Meads, M., Knight, V. H.,** and **Izlar, H. L., Jr.**: South. Med. J., **44**, 297, 1951.
23. **Szabo, J. L., Edwards, C. D.,** and **Bruce, W. F.**: Antibiotics & Chemotherapy, **1**, 409, 1951.
24. **Waldo, J. F.**: J. Omaha Mid-West Clin. Soc., **12**, 17, 1951.
25. **Waldo, J. F.,** and **Tyson, J. T.**: J. Lab. & Clin. Med., **37**, 272, 1951.
26. **Walker, W. F.,** and **Hunter R. B.**: Lancet, **2**, 106, 1951.
27. Iidem: Ibid., **1**, 104, 1952.

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PHILADELPHIA, PENNSYLVANIA

AND

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DETROIT, MICHIGAN

PRESERVATION OF WHOLE BLOOD AND RED CELLS

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THE transfusion of whole blood is now such a common practice that most younger medical practitioners do not realize how recent and important were the events which led to the establishment of the blood bank as a common hospital institution. Many of the older physicians and the relatives and friends of some former patients will recall the excitement attending the search for a suitable donor for the direct transfusion of a patient who was urgently in need of whole blood. Although the first blood transfusion[25] was performed as far back as 1665, it was not until the discovery of the blood groups in 1900 by Landsteiner[19] that the transfusion of whole blood became a relatively safe procedure. However, the modern era of the widespread use of whole blood for transfusion can be said to have started in 1916 when Rous and Turner[40] showed for the first time that whole blood could be satisfactorily preserved *in vitro* for two weeks or more. The first reported instance of the transfusion of preserved whole blood occurred during World War I and was the result of a British-American cooperative effort. Robertson[38], an American Medical Officer, working in British Army casualty clearing stations carried out 22 successful transfusions of blood which had been stored by the method described by Rous and Turner.

During the period between the two World Wars there was a rapid growth in the number of blood banks so that at the beginning of the last war the transfusion of preserved whole blood was a common procedure in medical and surgical practice. Because of the many logistical problems involved in supplying large quantities of whole

blood to distant areas of the world during war, considerable attention was devoted to the preparation and use of "blood substitutes". Although these "blood substitutes" are very useful and have been widely used, it has become increasingly evident that the expression "There is no substitute for whole blood" although trite is nevertheless true. There is probably no instance in which plasma or another "blood substitute" is superior in effect to that of compatible whole blood. There was and still is some feeling that in the severely burned patient it is safer to transfuse plasma than whole blood because of the hemoconcentration which occurs in this type of patient. However, this hemoconcentration is evidently more apparent than real for it has been shown[41] that in the severely burned dog the peripheral venous hematocrit is falsely high and is not a reliable indication of the requirements for red cells. Evans[5] concludes that "Whole blood is still the agent of choice for the initial treatment of the severely burned".

In the treatment of shock due to blood loss there is no question that whole blood is superior to plasma or any other agent. It is generally supposed that this superiority is due to the oxygen carrying capacity of the red cells. There is no doubt that the gaseous transport by the red cell and also its acid-base buffering capacity are important functions. However, Moore[32] has rightly emphasized the "space occupying" function of the red cell and it is this property which is perhaps its most important asset in the treatment of shock.

Whole blood is an indispensable agent for the treatment of the individual exposed to massive doses of ionizing irradiation. Although we fervently hope that nuclear warfare may never occur, the medical profession must know what to do if such warfare were to occur. The need for whole blood would be tremendous and would give great importance to the possibility of storing large quantities of whole blood or red cells for long periods of time.

Evaluation of Preserved Red Cells. The problem in the preservation of whole blood is mainly concerned with the viability of the formed elements, for it is well known that plasma, either wet or dried, can be stored without loss of osmotic efficiency for very long periods of time. The question of the viability of leukocytes and platelets for transfusion is at the present time an almost academic one, since it is doubtful whether these elements even if entirely uninjured will persist in the circulation for a significant time after transfusion[17,51]. Thus, the evaluation of the preservation of whole blood for transfusion purposes is reduced to a measurement of the viability of the red cells. A method of storage of whole blood which gives good preservation of the red cells can be said to be a good method of blood preservation.

This naturally poses the question of what constitutes good preservation of the red cell and how is it to be measured. From a functional point of view (and this is the only one that matters for transfusion purposes), well-preserved red cells are those which remain in the peripheral circulation after transfusion for their full normal life span. There is good evidence that nonviable red cells are removed from the circulation quite rapidly after transfusion[11]. It is therefore reasonable to conclude that those red cells which persist in the circulation are carrying on their normal functions.

The first direct method for measuring the persistence in the circulation of transfused red cells was devised by Todd and White[49] and might be called the method of differential hemolysis.

They were able to prepare a serum which selectively hemolysed the red cells of one bull but not those of another bull. Thus, after transfusion of one bull's blood into another, it was possible to hemolyze all the recipient's own red cells and to count the transfused cells in a specimen of the recipient's blood. This method has not been further developed and has not been reported in human studies.

A method using the same scheme but which is based on selective agglutination rather than hemolysis was first described in 1919 by Ashby[1]. In her early experiments she transfused group 0 blood to recipients of other blood groups and showed that the transfused cells could be counted as "unagglutinated corpuscles" after agglutination of the recipient's own red cells by treatment with a suitable serum. For example, if group O blood was transfused into a group A recipient, a post-transfusion sample of the recipient's blood was treated with a potent anti-A serum. This treatment caused the agglutination of the A cells so that the unagglutinated O cells (which had been transfused) could be counted. This same technique of differential agglutination has also been used with the M and N antigens[3,20] and with the Rh antigen[52]. There is still some difference of opinion about the precision obtainable with the Ashby method, but in experienced hands this is probably the best of the available methods for studying the survival of transfused red cells. The reader is referred to Mollison's excellent recent book[28] for a critical discussion of this question and also for the details of the technique.

Another method for studying the post-transfusion survival of red cells is one which utilizes radioactive iron[10]. In this method radioactive iron is injected into the donor and this element is then incorporated into the hemoglobin of newly formed red cells. The survival of these tagged cells in the recipient's circulation can then be followed by measuring the radioactivity of the red cells in samples of the recipient's blood after transfusion. This method suffers from the serious disadvantage that as the red cells are destroyed the liberated iron is re-utilized in the production of new red cells. Thus, except for some special cases[6], this method can be used to determine the survival of red cells for only a short time after transfusion.

Other radioactive isotopes have been used for this same purpose. Red cells can be tagged with radioactive phosphorus by a simple *in vitro* procedure[37]. Red cells so tagged have been used to study post-transfusion survival[44]. However, since there is fairly rapid exchange of phosphate between the red cells and plasma, it is possible to follow the survival of such red cells for only a few days. Radioactive chromium has also been used for tagging red cells *in vitro* and it has been shown that the chromium is firmly bound to the hemoglobin of the cell[14]. Although red cells tagged with radioactive chromium do not appear to survive or remain radioactive for their full normal life span[14], this method may prove to be useful in post-transfusion survival studies.

After reading thus far one should be impressed that the determination of whether or not red cells are viable and will remain in the circulation requires considerable time and effort and that it is not feasible to test many samples in a short time. Is there not a simple laboratory test which will reliably predict whether red cells will survive in the circulation after transfusion? Unfortunately, the answer to this question at the present time is "No". Many investigators have in the past used various *in vitro* tests as indications of red cell viability but it is now evident that none of them is reliable. Perhaps the

most widely used of these tests is the osmotic fragility test, in which the resistance of the red cell to hemolysis by hypotonic solutions is measured. Careful study has shown that osmotic fragility tests can be very misleading as a guide to *in vivo* survival[31]. Morphologic observations such as shape and dimension changes are also not useful guides. It was thought that a functional measurement might be a better answer to this problem. However, this is apparently not so, for it is possible to destroy the viability of rabbit's red cells without seriously impairing their glycolytic activity[18]. One can summarize the present usefulness of *in vitro* tests by saying that if by these tests a given sample of red cells shows significant deviation from normal then it is very likely that the cells are not well preserved. However, if the cells show no deviation from normal, no reliable deduction can be made as to their viability. *In vitro* tests are therefore useful as screening methods prior to direct measurement of viability.

CURRENT METHODS OF BLOOD PRESERVATION. Nearly all methods of blood preservation which are now in use involve the drawing of blood into a solution which acts both as an anticoagulant and as a medium to modify the environment of the red cells. The other important factor is the temperature of storage.

When Rous and Turner[40] preserved blood for the first time, they recognized the need for refrigeration and stored the blood at 2°C. A study[12] of the effect of storage temperature on viability indicated that the temperature range 4° to 10°C. was optimal. Thus it became general practice to store blood at approximately 5°C. It is not clear why the range 0° to 4°C. should be less favorable and indeed there is a growing opinion that storage at or near 0°C. is to be preferred[48]. The danger in routinely storing blood at 0°C. is that if temperature regulation is not very rigid and the temperature falls low enough so that freezing occurs, massive hemolysis may result. It is quite likely that significant deterioration of red cells occurs when stored blood is allowed to stand at room temperature for only several hours[39]. There is now increasing emphasis on the advisability of maintaining adequate refrigeration at all stages from the beginning of blood collection until transfusion[9].

Since Rous and Turner[40] first recommended the use of a preservative solution containing sodium citrate and dextrose, a tremendous amount of work has been done on the evaluation of different solutions as anticoagulants and red cell preservatives. The solution which is now in general use is one form or another of an acid citrate-dextrose solution which is commonly known as ACD[13]. It might be well to consider which factors it is important to regulate and to see how ACD accomplishes their regulation. The composition of one of the commonly used solutions which is known as ACD-3 is:

Trisodium citrate dihydrate	1.6 gm.
Citric Acid monohydrate	0.56 gm.
Glucose, anhydrous	1.5 gm.
Water to	100 cc.

Of this solution 15 cc. is used for each 100 cc. of blood.

One of the most important factors in red cell preservation is the pH of the fluid in which the red cells are suspended. The recognition that this pH should be slightly lower than that of the circulating plasma was an important advance in the field of blood preservation[22,23]. Maizels[27] has pointed out why an acid diluent is advisable. The results of several investigations indicated that the optimum initial pH for environment of the red cell is close to 7.2[21,33]. The ACD solutions themselves are considerably more acid than

this (about pH 6.0), but when added to fresh blood in the proper amount, the pH of the mixture is 7.2. It is the citric acid-citrate buffer system of ACD which is responsible for this regulation. Other preservative solutions[33] utilize a phosphate buffer system and are apparently just as efficient as ACD. In all cases the pH of the fluid portion of the blood decreases during storage, because of the lactic acid which is produced as the red cell utilizes glucose.

The ACD solutions provide adequate citrate to bind calcium and thus prevent clotting. However, this is apparently not the only function of the citrate. Since the red cell is not permeable to citrate, it certainly has some osmotic action, but perhaps even more important is the fact that citrate probably acts to prevent red cells from adhering to each other and forming large agglomerations[8].

Glucose has been shown to be an indispensable substance for preservation of the red cell for more than a few days. In an acid citrate medium without added glucose the red cells remain in good condition for a few days, presumably because of the glucose which was originally present in the blood. The rate of utilization of glucose by the red cells varies considerably with temperature, and thus the quantity of glucose which must be added to the blood will depend on the temperature of storage. The early ACD solutions yielded a final glucose concentration in the blood of about 550 mg. per 100 cc. More recently the tendency has been to use less glucose and in current practice a concentration of about 200 mg. per 100 cc. is used.

Another variable which has been studied is the extent of dilution of the shed blood. It was formerly thought that the addition of a rather large volume of preservative solution was beneficial to the red cell. This has been found not to be so[21,36], and since the commonly used ACD solutions are hypotonic, it is now felt advisable to restrict the volume of diluent.

There has been some difference of opinion as to whether the packed red cells of ACD blood are as well preserved after removal of the supernatant plasma as they are when no plasma is removed. An *in vitro* study[36] concludes that they are less well preserved, while an *in vivo* study[39] concludes that they store just as well. It seems reasonable to give greater weight to the opinion of the *in vivo* investigators.

Two of the more recent developments in the field of blood preservation relate to the means of preventing coagulation. The first of these is the decalcification of the blood by passage through an ion exchange resin. However, it seems that this treatment is detrimental to the red cell[4]. The other new development is the use of sequestrin, whose chemical name is ethylenediaminetetracetic acid. This substance is evidently an efficient and non-toxic anti-coagulant[35] but it is not yet clear what advantages it may have over citrate.

A large number of different substances have been proposed as agents to improve and prolong the preservation of red cells. In nearly all cases the evaluation of the effect of these substances has been by *in vitro* methods. Practically none of the claims made for them have been sustained when careful *in vivo* studies have been done. One recent case is illustrative of such a situation. It was reported[16] that the phenothiazine derivative known as RP.3300, which is closely related to the anti-histamine "phenergan", delays the appearance of spontaneous hemolysis and markedly prolongs the period during which the red cells retain their normal morphologic characteristics. Furthermore, red cells which had be-

come spherical after repeated washing in saline were rapidly converted to biconcave discs when immersed in very weak solutions of RP.3300. However, *in vivo* tests[2] showed that the addition of this compound to blood not only did not increase the storage life of red cells but appeared to be mildly deleterious. This case adds further emphasis to the truth of the assertion that *in vitro* tests are not reliable indications of post-transfusion survival.

Preservation at Temperatures Below Freezing. It has been well established that the life span of the human red cell in the normal individual is about 120 days. However, with our present methods of storage at a temperature some 30°C. below that of its normal environment, red cells can be preserved in usable condition for only about 3 weeks. Since the progressive loss of viability of red cells during storage is very likely due to the continuing metabolic activity of the cells, it seems reasonable to attempt to arrest metabolism by a still further reduction in storage temperature. However, if blood is taken below its freezing point, massive destruction of red cells occurs. Several attempts[7,15,26,47,50] have been made in the past to freeze and thaw red cells without destroying most of them, but some success has been achieved only with minute quantities of blood or very special conditions.

The discovery[34] that glycerol largely prevents the death and disruption of spermatozoa when they are subjected to temperatures well below freezing led to a chain of events which may in the near future revolutionize the practice of blood preservation. Subsequent to this initial discovery it was found[46] that red cells in the presence of about 15% glycerol could be frozen and maintained for a time at −79°C. (solid CO_2 temperature) with only slight hemolysis resulting after thawing. These thawed cells, since they contain considerable glycerol, are strongly hypertonic and hemolyze rapidly if placed directly into isotonic media. Accordingly, a procedure was developed[43] whereby the glycerol is slowly removed by dialysis and most of the red cells are recovered in apparently normal condition. The next link in this chain of events was the demonstration[44] that rabbit red cells which had been kept at −70°C. for periods up to 42 days survived in normal fashion when put back into the circulating blood stream.

The experiments described above were done with small quantities of blood, and so before human transfusion experiments could be carried out it was necessary to develop a procedure for freezing and recovering large volumes of red cells free from bacterial contamination. This was accomplished and it was then shown[29] that the freezing and thawing and storage for a few hours at −79° C. have no significant effect on the post-transfusion survival of human red cells. More recently it has been shown[30] that human red cells which have been stored for more than 8 months at −79°C. are capable of normal survival after transfusion. This was certainly good evidence that the metabolic activity of the red cell a −79°C. must be insignificantly small. Similar experiments were also carried out at −15°C. Although the proportion of red cells which could be recovered after storage at −15°C. was small, it was found that after 80 days at −15°C. those cells which remained intact had a normal post-transfusion survival[30]. This means that the metabolic activity of the red cell is very low at −15°C. and the long time preservation of red cells is feasible at this temperature which can easily be obtained with ordinary refrigeration equipment. Another advance which brings this method of preservation nearer practical application was the development of a method[24] by which

glycerol can be removed from red cells in a simple and rapid fashion.

Red cells which are stored by the present method at −79°C. undergo slow but continuous destruction[45], so that after 6 months about 70% of the cells can be recovered. The rate of destruction is more rapid at −15°C. The task which remains is to discover the cause of this destruction and to eliminate it. The likelihood that this task will be accomplished in the near future is good.

Sources of Blood. The prospect that it may soon be practical to store large quantities of red cells for long periods of time at low temperatures will probably not call for added sources of blood, since huge quantities of red cells are now discarded in the production of dried plasma. It is interesting, however, to note two sources of blood other than the volunteer donor. Blood taken from fresh cadavers has been stored and transfused[53]. A human placenta yields from 80 to 180 cc. of blood. Placental blood has been used for transfusion in a community where an adequate number of donors was not available because of war and malnutrition[42].

REFERENCES

(1) **Ashby, W.**: J. Exper. Med., **29**, 267, 1919.

(2) **Chaplin, H., Crawford, H., Cutbush, M.,** and **Mollison, P. L.**: J. Clin Path., **5**, 91, 1952.

(3) **Ebert, R. V.,** and **Emerson, C. P.**: J. Clin. Invest., **25**, 627, 1946. **(4) Emerson, C. P.**: in Transfusion Sanguine, p. 176, L'Expansion Scientifique Francaise, 1951. **(5) Evans, E. I.**: Symposium on Shock, Army Medical Service Graduate School, Washington, 1951.

(6) **Finch, C. A., Wolff, J. A., Rath, C. E.,** and **Fluharty, R. G.**: J. Lab. & Clin. Med., **34**, 1480, 1949. (7) **Florio, L., Stewart, M.,** and **Mugrage, E. R.**: Ibid., **28**, 1486, 1943.

(8) **Gengou, D.**: Ann. Inst. Pasteur, **79**, 628, 1950. **(9) Gibson, J. G.**: in Transfusion Sanguine, p. 161, L'Expansion Scientifique Francaise, 1951. **(10) Gibson, J. G., Aub, J. C., Evans, R. D., Peacock, W. C., Irvine, J. W.,** and **Sack, T.**: J. Clin. Invest., **26**, 704, 1947. **(11) Gibson, J. G., Peacock, W. C., Evans, R. D., Sack T.,** and **Aub, J. C.**: Ibid., **26**, 739, 1947. **(12) Gibson, J. G., Sack, T., Evans, R. D.,** and **Peacock, W. C.**: Ibid., **26**, 747, 1947. **(13) Gibson, S. T.**: N. Eng. J. Med., **239**, 544, 1948. **(14) Gray, S. J.,** and **Sterling, K.**: J. Clin. Invest., **29**, 1604, 1950. **(15) Griffets, J. J., Hornibrook, J. W.,** and **Downs, J. J.**: in Preservation of the Formed Elements and of the Proteins of the Blood, p. 167, American National Red Cross, 1949.

(16) **Halpern, B. N., Dreyfus, B.,** and **Bourdon, G.**: Presse Medicale, **58**, 1151, 1950.

(17) **Julliard, J., Maupin, B., Loverdo, A., Bernard, J., Colvez, P.,** and **Lecomte, M.**: Ibid., **60**, 518, 1952.

(18) **Kwiecinska, H. M.,** and **McKerns, K. F.**: Cited in the Preservation of the Formed Elements and of the Proteins of the Blood, p. 102, American National Red Cross, 1949.

(19) **Landsteiner, K.**: Wien. klin. Wchnschr., **14**, 1132, 1901. **(20) Landsteiner, K., Levine, P.,** and **Janes, M. L.**: Proc. Soc. Exp. Biol. & Med., **25**, 672, 1928. **(21) Loutit, J. F.**: J. Path. and Bact., **57**, 325, 1945. **(22) Loutit, J. F.,** and **Mollison, P. L.**: Brit. Med. J., **2**, 744, 1943. **(23) Loutit, J. F., Mollison, P. L.,** and **Young, I. M.**: Quart. J. Exp. Physiol., **32**, 183, 1943. **(24) Lovelock, J. E.**: Lancet, **1**, 1238, 1952. **(25) Lower, R.**: Philosophical Transactions, **I**, 352, 1666. **(26) Luyet, B. J.**: in Preservation of the Formed Elements and of the Proteins of the Blood, p. 141, American National Red Cross, 1949.

(27) **Maizels, M.**: Quart. J. Exp. Physiol., **32**, 143, 1943. **(28) Mollison, P. L.**: Blood Transfusion in Clinical Medicine, Blackwell Scientific Publications, Oxford, 1951. **(29) Mollison, P. L.,** and **Sloviter, H. A.**: Lancet, **2**, 862, 1951. **(30) Mollison, P. L., Sloviter, H. A.,** and **Chaplin, H.**: Ibid., **2**, 501, 1952. **(31) Mollison, P. L.,** and **Young, I. M.**: Brit. Med. J., **2**, 797, 1941. **(32) Moore, F. D.**: in Symposium on Shock, Army Medical Service Graduate School, Washington, 1951.

(33) **Parpart, A. K., Gregg, J. R., Lorenz, P. B., Parpart, E. R.,** and **Chase, A. M.:** J. Clin. Invest., **26,** 641, 1947. (34) **Polge, C., Smith, A. U.,** and **Parkes, A. S.:** Nature, **164,** 666, 1949. (35) **Proescher, F.:** Proc. Soc. Exp. Biol. & Med., **76,** 619, 1951.

(36) **Rapoport, S.:** J. Clin. Invest., **26,** 622, 1947. (37) **Reeve, E. B.,** and **Veall, N.:** J. Physiol., **108,** 12, 1949. (38) **Robertson, O. H.:** Brit. Med. J., **1,** 691, 1918. (39) **Ross, J. F., Finch, C. A., Peacock, W. C.,** and **Sammons, M. E.:** J. Clin. Invest., **26,** 687, 1947. (40) **Rous, P.,** and **Turner, J. R.:** J. Exp. Med., **23,** 219, 1916.

(41) **Salzberg, A. M.,** and **Evans, E. I.:** Ann. Surg., **132,** 746, 1950. (42) **Serafin, L.:** Sem. hôp. Paris **28, 654, 1952;** Int. Abst. Surg., **95,** 503, 1952. (43) **Sloviter, H. A.:** Lancet **1,** 823, 1951. (44) Idem: Ibid., **1,** 1350, 1951. (45) Idem: Nature, **169,** 1013, 1952. (46) **Smith, A. U.:** Lancet, **2,** 910, 1950. (47) **Strumia, M. M.:** Science, **110,** 398, 1949. (48) **Strumia, M. M., McGraw, J. J., Dolan, M.,** and **Colwell, L. S.:** Proc. Soc. Exp. Biol. & Med., **75,** 675, 1950.

(49) **Todd, C.,** and **White, R. G.:** Proc. Roy. Soc., B, **84,** 255, 1911.

(50) **Walter, C. W.,** and **Gibson, J. G.:** in Preservation of the Formed Elements and of the Proteins of the Blood, p. 169, American National Red Cross, 1949. (51) **Weisberger, A. S., Guyton, R. A., Heinle, R. W.,** and **Storaasli, J. P.:** Blood, **6,** 915, 1951. (52) **Wiener, A. S.:** Am. J. Clin. Path., **12,** 189, 1942.

(53) **Yudin, S. S.,** Lancet, **2,** 361, 1937.

OPHTHALMOLOGY

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RETINAL MICRO-ANEURYSMS

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IN 1945, I said[51], in a comment on the retinopathy of diabetes mellitus, "the earliest and the most characteristically diagnostic and therefore possibly the basic lesion is the small punctate hemorrhage in the presence of ophthalmoscopically normal retinal vessels." In 1943, Ballantyne and Loewenstein[10,11] had noted that the most characteristic hemorrhages in diabetes occur in or close to the macula as minute dark-red dots. They stated that when these minute lesions "present no central reflex, and have no visible connection with the blood-vessels, we may accept them as hemorrhages; but when they have a spherical form and are attached, as they often are, to the finest perimacular venules, like berries on a twig, we are probably dealing with aneurysms. We have no doubt that both lesions occur, but the differentiation of hemorrhages and aneurysms of such small size is not always possible by ophthalmoscopic examination alone, and, as we shall see, even microscopic investigation may leave us in some doubt."

In spite of the reports by Ballantyne and Loewenstein[10,11], I was still inclined to agree with Elwyn[18,19] that these so-called micro-aneurysms were merely irregular capillary dilatations and that the ophthalmoscopically visible red dots were actually hemorrhages, particularly in view of the rather large estimated size of the "aneurysms" (50 to 60 microns). I had seen occasionally groups of extremely fine red points, attached apparently to fine terminal venous twigs above the macula, that I designated "dilated capillaries"; it was estimated that they were much smaller than 50 microns. Hence, I agreed with Ballantyne and Loewenstein in thinking that the basic lesion responsible for the development of retinopathy in diabetes lay in the capillaries on the venous rather than on the arterial side of the circulation.

A disturbing feature of the small, punctate, so-called hemorrhages in diabetes had always been the tendency of some if not most of them to persist in the same location for long periods, even longer than indicated by Heath[27] when he said, "The startling thing about the punctate hemorrhages is that some dots disappear rapidly; others stay in the same place for several weeks." Ordinarily, such a minute hemorrhage should be absorbed in a few days. To explain this persistence of a red dot for months or even years, I was forced to assume that continued oozing or rebleeding occurred through damaged capillary walls. I had over-

looked the work of Nettleship[44] who, even before he published his descriptions of lesions of the larger veins and of vascular proliferation into the vitreous in diabetic patients, had reported finding capillary aneurysms in a microscopic study of the retinas of a 52-year-old woman who died in diabetic coma. In 1879, he, in association with Mackenzie[38], stated, "In the retina there are minute aneurysms on the capillaries. These are not very abundant; they often occur in little groups of about half a dozen or less near together, large adjoining tracts being quite free from them. They are globular pouches apparently communicating with only one side of the vessel, never fusiform dilatations. They usually contain some reddish-brown granules, and sometimes similar granules are seen lying in the tissue just external to them, suggesting that the bleeding into the vitreous may have been due to the consecutive rupture of a number of these minute sacs. The capillary walls are as a rule not much, if at all, thickened, but here and there considerable thickening is seen."

Since Ballantyne and Loewenstein[10,11] rediscovered the capillary aneurysms in the retinas of diabetic patients, rather extensive studies have been made, particularly microscopically, with regard to their occurrence in retinas in diabetes and in other diseases, to their pathogenesis and local mode of development and to the possibility of their presence in other organs and tissues. New techniques have been advanced for the demonstration of micro-aneurysms and for the study of their characteristics. They are known now to occur in conditions other than the retinopathy of diabetes, but why they appear more frequently and in greater numbers in diabetes than in any other disease is still not clear.

Histologic Techniques. The original studies of Mackenzie and Nettleship were made apparently on stained cross sections of the retina. Ballantyne and Loewenstein[10,11], in addition to an examination of ordinary sections stained with hematoxylin and eosin, studied the retinas in bulk, mounted in glycerin and viewed on the flat, both unstained and stained with scarlet red. They also injected the retinal vessels with a mixture of gelatin and blood and stained them with benzidine. Loewenstein[36] also stained the retinas in bulk with hematoxylin and Sudan. Evans[20] studied surface preparations of the whole retina stained with Weigert's stain for elastic tissue without counterstain. In his first work, Friedenwald[22] apparently used cross sections of the retina with ordinary stains. In 1948, however, he[23,24] made the first definite advance in the study of the nature of these microaneurysms when he adapted the staining technique of Hotchkiss and McManus for the demonstration of fixed carbohydrate. By this technique, only those places in the tissues that contain glycogen, mucoids or glycoproteins stain red. Friedenwald[23,24] found that in the retina the endothelium of the whole vascular tree (arteries, capillaries and veins) is surrounded by a brilliantly stained basement membrane. This membrane is continuous with the internal elastic lamella of the arteries. In arterioles that lack the elastic lamella, the basement membrane is prominently visible and appears to surround individual muscle fibers. In capillaries, this membrane is thinner than in arterioles but is still brilliantly visible. In venules it is thicker than in capillaries but is usually less thick than in arterioles of the same size.

With the aid of Becker and Day, Friedenwald[23-25] adapted this technique to the staining of unsectioned flat preparations of the whole thickness of the retina. Since the parenchyma of the retina normally contains no other

structures that stain by means of this technique, the whole retinal vascular pattern can be seen. Day[15] assumed that use of this technique allowed demonstration of polysaccharides in tissues. Becker and Post[14] used this same method in their studies, as did Wexler and Branower[53]. The latter authors, however, presented evidence to show that definite conclusions could not be reached as to the histochemical nature of the substances stained by the Hotchkiss-McManus method. In addition to the Friedenwald modification of the Hotchkiss-McManus staining method, Ashton[1-5] has used several techniques to study micro-aneurysms in bulk preparations of the retina: these include injection of India ink into the central retinal vessels; injection of the central retinal artery with red dye and the central retinal vein with black, to demonstrate that the aneurysms are confined largely to the venous side of the capillary system; and injection of the retinal vessels with neoprene followed by corrosion of the retinal tissues, to demonstrate that some of the aneurysms develop from vascular loops. Michaelson and Steedman[43] have described a method of injection of the retinal vascular system in enucleated eyes.

THE RETINAL CAPILLARY NETWORK. According to Michaelson and Campbell[42], the capillary system of the retina has a basic two-layered pattern. A superficial capillary net is present in the layer of nerve fibers and a deep capillary net lies in the boundary plane between the inner nuclear layer and the outer plexiform layer. Anastomotic capillaries run between the two nets. Both nets contain venous and arterial precapillaries, the venous being the larger. The deep net is denser and more complex than the superficial net. Poorer oxygenation of blood in the venous ends of the capillaries must be compensated for by denser distribution of capillaries in this region if the tissues are to be uniformly supplied with oxygen.

The general pattern becomes three-layered in the macular region, four-layered in the peripapillary zone and one-layered in the periphery of the retina. An avascular region measuring about 0.4 to 0.5 mm. surrounds the fovea. The deep capillary plexus is the most dependent part of the retinal circulation and in it the flow of blood is normally slower than it is in the more superficial capillaries. Smith[49] agreed with Michaelson and Campbell[42] that this anatomic arrangement is a factor in the development in diabetes of disproportionate metabolic disturbance in the capillaries of the deep retinal plexus, since the back pressure from venous stasis is most manifest there.

HISTOLOGIC CHARACTERISTICS OF MICRO-ANEURYSMS. In their first communication, in 1943, Ballantyne and Loewenstein stated that infiltration with fat in the endothelium of the capillaries appeared to be the earliest histologic evidence in the retina of diabetes. In some of the capillaries, the walls became ectatic, and formed actual aneurysms. The aneurysms appeared in two forms: (1) globular bodies obviously attached to vessels and often in series, like chains of beads, or (2) isolated spherical bodies consisting of closely packed erythrocytes enclosed within a capsule and without any discoverable connection with a vessel. They were located usually in the outer molecular layer. These investigators thought that the discrete spherical bodies represented the small round spots seen at the macula ophthalmoscopically as punctate hemorrhages and that histologically they were either encysted hemorrhages or aneurysms whose link with a parent vessel had atrophied or was too transparent to be seen.

In 1944, Ballantyne and Loewenstein[11] used the term "micro-aneurysms" and described them histologically as "perfectly round, mulberry-like collections of red blood corpuscles, enclosed in an envelope, sometimes represented by a thin structureless membrane, sometimes by a thicker wall of double contour, and sometimes by a wall of considerable thickness." They stated that these micro-aneurysms were located on the capillaries which linked the superficial capillary network in the layer of nerve fibers with the deeper plexus at the outer boundary of the inner nuclear layer; they might be seen therefore in the inner nuclear layer, the layer of ganglion cells or the layer of nerve fibers and more rarely in the outer molecular layer. Occasionally marginal extravasation of blood was seen as a result of diapedesis through or rupture of the dilated capillary wall. The size of these micro-aneurysms varied from 30 to 90 microns, the majority being about 50 to 60 microns. In some instances, the endothelial lining of the capsule of the micro-aneurysm appeared to have proliferated and swelled, with resultant thrombosis of the aneurysm and formation of a cicatricial nodule. These authors stated also that the globular micro-aneurysms were only one variety of the ectatic lesions that occur on retinal capillaries. Sometimes relatively long segments of the capillaries were so dilated as to form sausage-like loops or complex coils or networks partly obscured by hemorrhage. In 1945, Ballantyne[8] added to these descriptive data the observations that the walls of the aneurysms had at times a fibrillary character, that the micro-aneurysms were situated between the precapillaries on the arterial and those on the venous side of the retinal circulation and that this location indicated that the initial pathologic change was on the venous rather than on the arterial side.

In his first publication on the subject of micro-aneurysms in 1947, Friedenwald[22] stated that the aneurysmal sacs were often incompletely lined with endothelium; in his opinion this indicated that leakage of blood occurred first from a break in the capillary wall and that later endothelial cells grew out around the mass of extravasated erythrocytes, with eventual formation of a minute saccular aneurysm. He thought that the presence of generalized increase in capillary fragility in diabetic patients who had retinopathy favored this mode of development of the aneurysms. In later publications, however, Friedenwald[23,24] presented proof that the localized dilatations (which he had first mentioned in 1936 in an unpublished paper) had both afferent and efferent connections to the parent capillary and were, therefore, true aneurysms and not endothelialized petechiae, which would be connected to the vascular tree by a single channel. He noted that in diabetic retinopathy these micro-aneurysms were regularly present by dozens or hundreds and that innumerable ones were present in the retinas in every case of Kimmelstiel-Wilson's nephropathy that he studied. He[25] examined histologically the retinas of 76 diabetic patients and found capillary aneurysms in 43 (57%); these were mainly in the region about the disks and maculas, but a few were located peripherally. The average duration of the diabetes was 5 years in the cases in which aneurysms were absent and 13 years in the cases in which aneurysms were present. He thought that the aneurysms were mostly spherical in shape and were probably globular from the beginning. Most of them were about 20 to 30 microns in diameter, but occasional aneurysms were as large as 80 to 100 microns. In his opinion, most of them

were too small to be seen ophthalmoscopically and, therefore, many early stages and mild lesions might be missed clinically[26]. Both Friedenwald[23-26] and Ashton[3,5] noted the hyaline thickening of the walls of many of the micro-aneurysms that served perhaps to prevent their rupture.

Ashton has made extensive investigations of micro-aneurysms in the retinas of diabetic and nondiabetic persons and also in other tissues of diabetic patients. By methods utilizing injection, he was able to demonstrate conclusively that the dilatations were on the venous side of the capillary network and, therefore, strictly speaking, were not aneurysms. However, the term "capillary micro-aneurysm" has been generally accepted to designate this lesion. He noted that in most instances the dilatation arises from one side of the vessel, the other side appearing perfectly normal. He stated, in 1951[5], that he had examined postmortem material, including the eyes, from 121 patients who had diabetes. In 110 of these, the data were sufficiently complete to allow analysis. Retinopathy in various stages (and presumably micro-aneurysms) was present in 68% of the cases; Kimmelstiel-Wilson's disease was present in 36%. In every case of Kimmelstiel-Wilson's disease, micro-aneurysms were present in the retina. Ashton's[5] estimates of the size of the micro-aneurysms agreed with those of Friedenwald[25] in that they were usually 20 to 30 microns in diameter but ranged up to 75 or 100 microns. Ashton[5] thought that the aneurysms might become completely obliterated and form at least some of the white dots seen ophthalmoscopically.

Local Mode of Development of Micro-aneurysms. Ballantyne[8] considered that two factors appeared to be requisite for the formation of these aneurysms, namely, weakness of resistance of the capillary wall and relative increase of pressure within the vessel. Swelling and fatty degeneration of the endothelial cells of the capillaries might partially or completely block the lumen and also weaken the walls so that they would yield to the endovascular pressure. He thought that stasis of blood would further damage the endothelial cells and favor progressive increase in size of the aneurysmal dilatation. Loewenstein[36] thought that mechanical factors (venous stasis?) were largely responsible for the development of the micro-aneurysms. Friedenwald[25] stated that, since aneurysms are not seen in newly formed retinal capillaries, increased capillary pressure is not in itself sufficient to cause the dilatations. Some abnormal local weakness of the wall appears to be essential. Whether the disturbance of the polysaccharide-containing portion of the capillary wall mentioned by Day[15] is responsible for this weakness in the wall, or whether it is anatomic at points of bifurcation or sharp bending of the capillaries, is not clear.

Friedenwald[25] noted also that the terminal loops of newly formed vessels in the vitreous show bulbous ends resembling intraretinal capillary aneurysms. In Elwyn's opinion[18,19], dilatation of the capillaries (micro-aneurysm) is a manifestation of prestasis resulting from contraction or relative narrowing of the parent artery or precapillary arteriole. Ashton[5] appeared to think that the aneurysms developed largely as a result of venous stasis, though it was possible that they might arise, especially in nondiabetic persons, in focal areas of degeneration in the vessel wall, which might in turn be caused by local anoxia. The basement membrane described by Friedenwald[23,24] appeared to play an important part in the pathologic process; sometimes it was greatly thickened, at other times it apparently split and disinte-

grated at the edge of the aneurysm. In Ashton's[5] opinion, some aneurysms developed by means of gradual enlargement of minute diverticula arising from one side of the capillary wall. More frequently, however, they apparently originated in loops of capillaries that became varicose and then were sealed together by exudate that had oozed through the capillary wall. This mode of development would explain the presence of a large aneurysm on a capillary that otherwise appeared normal.

In his study of flat preparations of the retina, Evans thought that he could demonstrate the presence of sphincteric muscle at the point of junction of small retinal capillaries with those of the next greater size. He surmised that prolonged constriction of these sphincters might be responsible for development of the capillary aneurysms observed in cases of absolute glaucoma. Evans did not state definitely whether these aneurysms were in arterial or in venous capillaries. Lawrence[35] thought that the "necessarily high pressure in the venous ocular system" must contribute to vascular degeneration in retinas of diabetic persons. Fraser, Simpson and Ashton[21] also noted that venous pressure must be greater in the eye than elsewhere in the body because it must be at least a little greater than intra-ocular tension. A decrease in intra-ocular tension associated with an increase in value for blood sugar might diminish the support of the retinal vessels and thus be a factor in the development of capillary aneurysms. Bair[7] has estimated that normally the retinal venous capillary pressure should be about 10 to 15 mm. of mercury more than the intra-ocular tension; thus, it should average at least 25 to 30 mm. of mercury, which is considerably greater than the normal pressure in peripheral veins. The dilatation of the veins in the retinas of patients who have diabetes suggests a pathologic increase in venous pressure. Ashton[3] suggested that the answer to aneurysmal formation and so to diabetic retinopathy was to be found in the solution of this problem of increased venous pressure. Fraser, Simpson and Ashton[21] thought that comparison of the mechanical and chemical factors operating in the eye and in the glomeruli of the kidney might aid in determining the pathogenesis of the lesions.

Retinal Micro-aneurysms in Non-diabetic Patients. The finding of numerous micro-aneurysms in microscopic studies of retinas of diabetic patients led to a similar search for them in normal and in diseased retinas of persons who were known not to have diabetes. In most of such retinas in which micro-aneurysms were found, obvious gross causes of venous stasis were present. Ballantyne[8,9], Loewenstein[36], Ballantyne and Michaelson[12,13], Loewenstein and Garrow[37], Friedenwald[25], Becker and Post[14] and Ashton[3-5] all mentioned the presence of micro-aneurysms in cases of thrombosis of the central retinal vein or one of its tributaries.

Becker and Post[14] examined flat preparations of the retinas of 39 eyes in which occlusion of the central vein was present and found micro-aneurysms in all. Most consistent and typical were chains of aneurysms on the venous side of the capillary circulation. Sometimes isolated, thick-walled, saccular aneurysms were present that were similar to those found in diabetes. Hemorrhages and exudates surrounded and were closely associated with the aneurysms. By application of diathermy to optic disks of cats by means of a 120-micron wire electrode introduced under ophthalmoscopic observation, Becker and Post[14] produced the clinical picture of occlusion of the central vein. In both flat and serial-section

preparations of these retinas, they were able to demonstrate hemorrhages, exudates, venous thrombi, isolated capillary aneurysms, chains of aneurysms at the venous ends of capillaries, distortion of capillary architecture and newly formed vessels. They stated, "Paravenous chains of saccular capillary aneurysms are demonstrated in cases of central vein occlusion and of Eales' disease which simulate those of diabetic retinopathy but, on the whole, differ in the part of the capillary affected, the location in the retina, and their general appearance." The occurrence of retinal micro-aneurysms in Eales's disease (periphlebitis retinae) has been reported also by Ballantyne and Michaelson[12] and by Loewenstein[36]. The latter author mentioned seeing them also in a case of retinitis circinata and frequently in senile retinas with arteriosclerosis; he stated that the micro-aneurysms in venous thrombosis were usually larger than those in diabetic retinopathy.

Wexler and Branower[53] examined bulk preparations of the retinas of 53 patients; 13 of these had diabetes and 40 had hypertensive vascular disease. Eleven of the 40 in the latter group had malignant nephrosclerosis and significant capillary lesions were present in all; the remaining 29 had nonmalignant hypertensive disease and significant capillary lesions were found in only one. In the retinas of all the diabetic patients, micro-aneurysms and sacculations of the capillary walls were found, predominantly in capillaries closely connected with small venules. The retinas of all eyes in cases of the malignant phase of hypertension and of pathologically proved malignant nephrosclerosis exhibited dilatation and sacculation of some of the arterial capillaries and focal reduplication of the basement membrane at the point of sacculation. The authors considered that various stages in the growth and development of this reduplication or thickening led to the formation of a lesion in the capillary wall resembling the micro-aneurysm of diabetes. This would suggest a specific disturbance in the arterial capillary circulation contrasting with the lesion in the venous capillary circulation in diabetes.

In 1950, Friedenwald[25] said that an appreciable number of retinal micro-aneurysms was found in only one of more than 76 nondiabetic persons. In only 3 of 13 cases of hypertensive retinopathy, was the presence of one or 2 isolated micro-aneurysms noted. Aneurysms were not found in elderly arteriosclerotic patients, regardless of the presence or absence of retinitis. However, in 1952, commenting on the findings of Wexler and Branower[53] in malignant hypertension, Friedenwald[26] said, "My own experience is that they occur most frequently in patients of this group who have arteriolonecrotic nephritis, and they are, in general, located at the margins of focal retinal lesions." He[25] stated also that aneurysms in the walls of venules and in the capillary bed in cases of venous occlusion differ in morphology from those encountered in diabetes.

Day[15] stated, "The clinical impression that these aneurysms are characteristic of chronic diabetes is not entirely borne out by the study of autopsy material"; he was of the opinion that occasionally a few aneurysms might be found in cases of chronic hypertension. Also Igersheimer[30] said that aneurysms are seen in nondiabetic diseases, notably malignant hypertensive and other retinopathies, but that in these cases they are rare and occur in small numbers whereas in the retinopathy of diabetes they may be innumerable. Sysi[50] searched for micro-aneurysms in the retinas of 143 pairs of eyes. He found aneurysms in cases of generalized arteriosclerosis, of nephrosclerosis and of nephritis. No aneu-

rysms were present in the eyes in 51 cases classified as controls. Micro-aneurysms were demonstrated in 10 of 23 cases of generalized arteriosclerosis (44%), in 18 of 47 cases of nephrosclerosis (38%) and in 9 of 22 cases of nephritis (41%). Two main types of aneurysms were seen, namely, a localized spherical type and an elongated, fusiform, varicose type. No discernible difference was present among the aneurysms in the different diseases. They were located both at points of vascular bifurcation and in the segments between bifurcations. Aneurysms were fairly equally distributed among patients of various age groups, although the incidence tended to increase with advancing age in patients who had nephrosclerosis and to decrease with advancing age in patients who had arteriosclerosis.

Ashton[3-5] has used both staining and injection techniques in his extensive investigations of the occurrence of retinal micro-aneurysms in nondiabetic persons. In his report to the Sixteenth International Congress of Ophthalmology, published in 1950[3], he stated that capillary aneurysms were not present in clinically normal eyes even in persons of advanced age, nor had he found them in persons who had benign hypertension. He had encountered micro-aneurysms in patients who had thrombotic glaucoma or chronic iridocyclitis with secondary glaucoma; he also found them in one patient who had a melanoma of the choroid and in whom the tumor had obstructed the central vein. However, he added in a footnote that further studies of eyes from nondiabetic persons had shown that "the retinal microaneurysm is a very common lesion. In most cases its formation may be explained by vascular degeneration associated with prolonged venous engorgement and/or a loss of external support through variations in intra-ocular pressure."

In papers published in 1951, Ashton[4,5] stated that he had examined histologically 336 eyes of persons who did not have diabetes mellitus. Micro-aneurysms were found more frequently by injection than by staining techniques. Of 135 eyes removed by enucleation during life, the retinal vessels were satisfactorily demonstrable in 89; in 30 (34%) of these, micro-aneurysms could be seen. The cases in which micro-aneurysms were found included 10 of thrombotic glaucoma, 10 of glaucoma secondary to chronic uveitis, 2 of chronic simple glaucoma, 3 of chronic uveitis and one each of sympathetic ophthalmia, Coats's disease, retinoblastoma, melanoma of the choroid and glaucoma secondary to a perforating wound of the globe.

He studied 174 eyes that were removed at necropsy; all of these were examined by injection techniques. Micro-aneurysms were found in the retinas of 29 of the 85 persons on whom the report is based (34%). In 6 of these, retinal lesions would probably have been visible during life. Three of these 6 had retinopathy of malignant hypertension, one had polycystic disease of the kidneys, one had proliferating retinitis with detachment of the retina and the other had hypertensive disease. Micro-aneurysms were present in all 6 of these persons and also in 23 (29%) of 79 persons in whom no ocular disease was suspected during life and in whom the eyes might be considered to be normal. The aneurysms were confined to the peripheral part of the retina in 10 of these cases (44%) and to the posterior part in 4 (17%); whereas they were scattered throughout the retina in 9 cases (39%). Evidence of intercapillary glomerulosclerosis was not present in any of these 23 cases. In "normal" eyes, the micro-aneurysms were usually small and few in number. They resembled those encountered in diabetes; some were beaded, and

some arose from one side of the vessel wall or from adhesion of varicose loops. They varied from 10 to 50 microns in size. In 2 of the 3 cases of malignant hypertensive retinopathy, Ashton[4] found micro-aneurysms confined to the venous side of the capillary network that were exactly similar to those observed in diabetes; in the third case, micro-aneurysms were present on both the venous and arterial sides of the capillary network. He thought that this was explained by the fact that the micro-aneurysms were caused by a decrease in oxidation and nutrition of the vessel walls secondary to chronic arteriospasm.

Ashton[4] noted further that the incidence of micro-aneurysms was greater when they were searched for by means of injection techniques than when staining methods were used. He thought that this was accounted for by the superiority of injection techniques, since he was convinced that "it is not possible to produce aneurysms as an artefact by the injection method." He[5] stated, "It is clear that, apart from vascular sclerosis, the occasional capillary micro-aneurysm is the commonest pathological lesion in the retina"; and "It is difficult to elaborate a single theory which will explain the histogenesis of these lesions in each of the conditions in which we now know them to occur." He[4] apparently thought that toxic damage to the capillary walls, as in uveitis, and circulatory stasis were the most common factors in the production of micro-aneurysms in "pathologic" retinas. He[4] had no explanation for their occurrence in "normal" retinas, since they appeared not to be related in any definite way to the age of the person, to the blood pressure during life or to the cause of death.

Micro-aneurysms in Other Tissues of Diabetic Patients. Day[15] made the statement that the "capillaries of the retina are selectively though not exclusively involved in the aneurysm-producing phase of diabetes." He called attention to the fact that a few lesions resembling retinal micro-aneurysms might be seen in the capillaries of the fold of the fingernail and in the brains of persons who had the retinopathy of diabetes. In 1950, Friedenwald[25] stated that he had found only one aneurysm during slit-lamp studies of the conjunctivas of 60 diabetic patients who had retinopathy. He had not found aneurysms in the microscopic study of ocular tissues other than the retina. Among 40 cases of diabetic retinopathy, he had found clusters of aneurysms in the nail-fold capillaries of 3 cases. (Weinstein[52] stated that aneurysmal dilatations occurred in the nail-fold capillaries in all cases of diabetic retinopathy.) Friedenwald[25] had found one aneurysm in the examination of the brains of 10 persons who had diabetic retinopathy.

In 1952, Friedenwald[26] reported the incidence of saccular aneurysms in the conjunctival capillaries of diabetic patients who had retinopathy to be about 5%, which he thought was somewhat greater than the incidence in nondiabetic persons. He considered that the estimates of McCulloch and Pashby[41] were too high. These latter authors stated that they had made slit-lamp studies under high power of the conjunctivas of 99 diabetic patients and 52 nondiabetic persons in a search for capillary aneurysms; they had found them to be present in 55% of the patients who had diabetes and in 14% of the nondiabetic persons. Two types of aneurysms were seen, namely, a true berry type and an aneurysmal dilatation of the veins that was not considered to be of clinical significance and was not included in the statistical analysis. The berry-type aneurysms resembled those noted in the retina; they were purplish, had sharp borders and round, smooth surfaces and might or

might not have visible connection with a capillary. They occurred in any part of the conjunctiva and might be superficial or deep in the conjunctival stroma. They remained unchanged as to size, shape and position during a period of several months. Berry-type aneurysms were present in 34 of 55 diabetic patients who had retinopathy (62%) and in 21 of 44 diabetic patients who did not have retinopathy (48%). McCulloch and Pashby[41] regarded these lesions as characteristic of diabetes and suggested, partly on this basis, that diabetes is a general vascular disease and that its variable manifestations depend on the degree of involvement of vessels in different parts of the body.

Ashton[5] made extensive studies of various tissues and organs to determine whether micro-aneurysms occur in diabetic patients anywhere except in the retina. He noted that it was difficult to find micro-aneurysms in sections of tissue, even in serial sections. In a study of tissues from about 30 diabetic patients, he made flat preparations of the serous membranes of the pleura, meninges, kidneys and liver to obtain views comparable with those of flat preparations of the retina. The brains, livers, kidneys and lungs were examined by injection techniques followed by clearing of thick slices by means of xylol. Ashton[5] examined the vessels of all other parts of the eyes. He injected India ink into the gangrenous leg removed from a diabetic patient and examined the whole capillary network in serial slices. Aside from those in the retina, he found no aneurysms except in the glomeruli of the kidneys. He stated that he did not consider conjunctival tissue to be a suitable site for study of extra-ocular capillaries, for it is exposed and subject to recurrent minor traumas.

Ashton[1-5], Day[15,16] and Friedenwald[23-26] have all called attention to the frequent association of intercapillary glomerulosclerosis with the retinopathy of diabetes and to the microscopic similarity between retinal micro-aneurysms and the nodular renal lesions. Friedenwald[25] stated that Day had found that 25 (58%) of 43 diabetic patients who had retinal micro-aneurysms had typical nodular deposits of hyalin in the renal glomeruli. Ashton[5] reported that 40 (53%) of 75 patients who had diabetic retinopathy had Kimmelstiel-Wilson's disease and that of 17 patients who had exhibited retinopathy graded 4, 16 (94%) had Kimmelstiel-Wilson's disease. It was stated by Day and by Ashton[5] that retinal micro-aneurysms could be demonstrated in all of 25 and 40 cases respectively of proved Kimmelstiel-Wilson's disease. Day[15] stated that good presumptive evidence exists that the basic retinal and renal lesions in diabetes are identical. Ashton[5] demonstrated aneurysmal dilatations in association with some of the nodular masses of intercapillary glomerulosclerosis and thought that the histogenesis of the glomerular lesion was in every way comparable to the pathologic process in the retina. He said[3], "By indian-ink injections of the kidney followed by Masson's stained sections we have now been able to demonstrate micro-aneurysms in the glomerular tufts in Kimmelstiel-Wilson's disease. . . . The intercapillary hyaline masses are seen to be closely associated with these aneurysmal dilatations; there are hyaline thickening of the vessel wall and exudate formation. The pathological appearances resemble a diabetic retinopathy in miniature."

In his latest paper on diabetic retinopathy, Friedenwald[26] again called attention to the close connection between retinal micro-aneurysms and the renal lesions of diabetes. He agreed with Ashton[1,5] that no distinguishing histochemical or staining characteristics existed between the hyalin of the

nodules in Kimmelstiel-Wilson's disease and that in the thickened walls of the retinal capillary aneurysms. He was inclined to think that the hyalin was laid down inside the basement membrane of the retinal aneurysm, whereas Kimmelstiel thought that in the kidney the hyalin was laid down outside the basement membrane and was not necessarily related to a pre-existing aneurysm. Friedenwald[26] stated, "The evidence seems unequivocal and complete that the retinal aneurysms and the Kimmelstiel glomerular nodules are joint manifestations of the same disease process." He was of the opinion that this process is characteristic for diabetes and is not dependent in any way on hypertensive disease or on atherosclerosis.

Pathogenesis of Retinal Micro-aneursym in Diabetes. The facts that, if their possible presence in the kidneys is excepted, micro-aneurysms are found in diabetes practically only in the retina and that micro-aneurysms are found in the retina in diseases other than diabetes and even in "normal" eyes, would suggest that factors peculiar to the retinal circulation are responsible for the formation of micro-aneurysms and, therefore, they cannot be considered to be characteristic of diabetes. However, when it is borne in mind that in most if not all instances (except in diabetes) in which micro-aneurysms are seen in the retina, at least ophthalmoscopically, other gross pathologic changes are visible, it appears justifiable to agree with Friedenwald that, "It is difficult to avoid the conclusion that in the diabetic the aneurysms represent a primary vascular lesion in the retina while in other diseases the aneurysms occur in response to local injury." Also, if it is admitted that micro-aneurysms occur in the conjunctiva and in the kidneys of diabetic patients, then it can be assumed, as Ashton[3] thinks, that the micro-aneurysms are probably simply the expression of a generalized vascular disease modified by local mechanical factors. A solution to the pathogenesis of the retinal micro-aneurysms may then lead to a solution of the whole problem of vascular degeneration in the person who has diabetes. It is this fact that makes intensive study of the nature of retinal micro-aneurysms worth while.

The assumption by Ballantyne[8,9] and Ballantyne and Michaelson[12,13] of the presence of an unknown circulating chemical toxin that is highly selective for the retinal veins and for the venous side of the capillary network appears extremely speculative, as do their suggestions of a deficiency in the blood of diabetic patients of certain constituents requisite for the nutrition of capillary endothelium or of the presence in the retina of locally produced abnormal metabolites specific to diabetes. The same conclusion applies to Sysi's[50] postulation of a disorder in the central regulatory mechanism common to arteriosclerosis, nephrosclerosis and diabetes.

It may be more logical to assume that the fatty degeneration of the endothelial cells of the capillaries observed by Ballantyne[8,9] is secondary to anoxia resulting from venous stasis than that it is the primary cause of the micro-aneurysms. Venous engorgement or stasis has long been recognized as one of the ophthalmoscopic features of the retinopathy of diabetes; attempts at therapy of the retinopathy with Dicumarol have been based on the assumption that the hemorrhages result from obstruction to the return flow of venous blood from the retina. Since occlusive lesions have not been demonstrated in the central retinal vein, it has been presumed that the stasis has its origin in the capillary system itself. According to Ashton[1,3], Cristini and Tolomelli claimed that

sclerosis of the precapillaries in the retina, stasis and increase in venous pressure occur in diabetic patients. However, Ashton[3] stated that sclerosis of the precapillaries may or may not be present in the retina of persons who have diabetes and sclerosis of these vessels may be seen in patients who have arteriosclerosis without associated micro-aneurysms.

Elwyn[18,19] also stressed the slowing of the flow of blood through dilated terminal vessels induced, he thought, by prolonged hyperglycemia. The idea that hyperglycemia itself is the main factor causing the retinal lesions gains some support from Martin's[40] observations of the much wider variations noted in intra-ocular tension during a 24-hour period in diabetic persons than in normal persons (which may be a factor in the local development of micro-aneurysms) and also from recently published data on the relative frequency of retinopathy in well-controlled and poorly controlled diabetes. Scott[47] classified as "defaulters" those patients who had diabetes and who did not attend the follow-up clinic regularly. Forty-five of 62 patients who had retinopathy (73%) were defaulters, whereas only 6 of 34 patients (18%) who were free from all signs of degenerative disease were defaulters. Keiding, Root and Marble[33] confirmed the relationship between the duration of diabetes and the incidence of retinopathy but found that duration of diabetes was not the sole deciding factor. They stated, "Degree of control of diabetes over the years is of paramount importance. No patient with excellent control had more than minimal (grade 0 or 1) eye changes."

Sherrill[48] also expressed the opinion that retinal changes in diabetes could be retarded for an indeterminate number of years by proper regulation of the disease, and that such changes appeared to be activated by the processes that accompany uncontrolled diabetes. However, he called attention to the fact that diabetes involved more than metabolism of carbohydrates. Jacobs[31] stated that glucosamine is thought to occur in the blood in the form of a polysaccharide bound to a protein molecule of the plasma, and that the amount of plasma glucosamine follows the values for blood sugars. He thought that the mucopolysaccharide of the mucoid fraction of the blood plasma was involved in the defect of diabetes and that it responded to insulin in roughly the same manner as glucose itself. The previously mentioned experiences of Friedenwald[25,26] and of Day[15] with the Hotchkiss-McManus staining technique led these workers to suggest that increased capillary fragility and retinopathy in diabetic patients were related to this disturbance in the metabolism of mucopolysaccharides and to an alteration of the polysaccharide-containing portion of the capillary wall. Ashton[5] was inclined to agree that "the muco-polysaccharide protein complexes within the vessel walls are in some way involved in the diabetic defect." He said, however, "It is too early to hazard a guess as to why these mucoids should be withdrawn from the vessel walls in some areas and deposited in others, as in the glomerulus."

More recently, Friedenwald[26] called attention to the fact that deposits of hyalin indistinguishable from those in diabetes occurred sometimes in the walls of the retinal capillary aneurysms found by Becker and Post[14] after occlusion of the retinal veins in experimental animals. He said, "It would appear that no abnormal plasma mucoprotein is required for the accumulation of the hyalin deposit, but the possibility that some abnormality of the mucoid metabolism of the diabetic may underlie the process has not been excluded." In 1945, Schneider and his

co-workers[46] reported that hemorrhages occurred in the retinas of alloxan-diabetic rabbits if the serum proteins were decreased. They did not mention micro-aneurysms.

In 1951, Day[16] stated that he had been unable to demonstrate characteristic micro-aneurysms in animals that had experimentally induced diabetes. Janes and associates[32], in their report on alloxan diabetes in the rat, stated that they did not observe changes typical of diabetic retinopathy but they had not completed histologic studies of the eyes. In his most recent paper, Friedenwald[26] called attention to the facts that administration of cortisone to rabbits by Rich and his co-workers resulted in lesions that simulated the nodules of Kimmelstiel-Wilson's disease and that Rich had found typical lesions of Kimmelstiel-Wilson's disease in the kidneys of a nondiabetic person after prolonged intensive treatment with corticotropin (ACTH). In collaboration with Becker*, Friedenwald[26] has been investigating the possible role of adrenal cortical function in the production of capillary lesions in diabetic persons. He stated, "We find nothing in the literature or in our own clinical and experimental studies that contradicts the hypothesis that the vascular lesions of the diabetic may be due to some interaction between the diabetic metabolic abnormality and adrenal cortical function." Friedenwald[26] and Becker* have studied the retinas and kidneys of alloxan-diabetic animals treated with cortisone and corticotropin and have compared them with those of alloxan-diabetic animals that did not receive hormonal therapy and of control animals that did not receive alloxan but that did receive hormonal therapy. They were able to demonstrate renal lesions in the alloxan-diabetic animals that received hormonal therapy that were similar to those described by Rich; they also found retinal capillary aneurysms similar to those encountered in diabetic patients. Friedenwald[26] admitted that, "It is too early to conclude that the lesion is the result of some synergism between the diabetic state and adrenal cortical hormones." He said also, "Whatever the fate of our present working hypothesis, however, it is clear that this experimental analogue of the diabetic vascular lesion merits further study."

OPHTHALMOSCOPIC DIAGNOSIS OF RETINAL MICRO-ANEURYSMS. Since it has been rather clearly demonstrated by various histologic techniques that many if not all of the so-called punctate hemorrhages noted in the retinas of persons who have diabetes are actually micro-aneurysms, that the micro-aneurysms most probably precede the hemorrhages and that they are the initial phase of a usually progressive retinopathy, it would appear to be of little moment to attempt to differentiate ophthalmoscopically between the two lesions. However, the differentiation is of some importance in evaluation of the effects of any type of therapy in the stage when these so-called punctate hemorrhages alone are present. True punctate hemorrhages might be expected to disappear rather rapidly and not to recur if treatment is efficacious, while micro-aneurysms could be expected to disappear if at all only after a prolonged period of observation. Personally, I have not been sure that I can definitely differentiate the two lesions on primary examination. In my opinion, the persistence of the red dot in the same location and in the same

* Dr. Bernard Becker (Ann. Int. Med., 37, 273, 1952) gave more details of the study of the role of adrenal cortical hormones in the development of retinal micro-aneurysms in experimental diabetes and noted also that he had found micro-aneurysms in the retina ophthalmoscopically in pernicious anemia and in 2 cases of sarcoidosis treated with corticotropin (ACTH), and histologically in some cases of adrenal hyperplasia.

size and shape for periods of months and years has been the most accurate distinguishing feature. If this criterion for diagnosis is accepted, then it becomes obvious that many of the so-called punctate hemorrhages of the diabetic patient actually are micro-aneurysms; the same conclusion applies to many of the numerous punctate hemorrhages observed in the residual phases of closure of the central or tributary retinal veins and also to some of the isolated "hemorrhages" noted occasionally in the retina in association with healed choroiditis or arteriolosclerosis or even in otherwise apparently normal fundi. Thus, Ashton and Langley[6] described capillary micro-aneurysms associated with macular and peripheral choroidoretinal degeneration in a 37-year-old man, and Edington and Sarkies[17] reported the presence of isolated micro-aneurysms in the retinas of 2 patients who had sickle cell anemia.

Most observers think that some of the red dots in the retinas of persons who have diabetes are actual hemorrhages and some are micro-aneurysms, although most if not all of the truly punctate lesions are micro-aneurysms. They agree that ophthalmoscopic differentiation between small hemorrhages and micro-aneurysms is not always possible. The distinguishing features listed by various observers are so nearly identical that it would appear sufficient to quote the differential points listed originally by Ballantyne and Loewenstein[10,11] and amplified later by Ballantyne[8,9]. These investigators noted that the fine capillary aneurysms were limited in size (usually 20 to 60 microns) and that they maintained this same size for 3 to 4 years at least. They were perfectly round in shape and sometimes were shown to be globular by the presence of a central light reflex. They were sharply defined because of their relatively superficial situation in the inner nuclear, inner molecular and ganglion-cell layers. Occasionally they appeared to be attached to the finer twigs of the small perimacular vessels, although actually they might not be so attached. A faint halo might develop around some of them and this might gradually become whiter and broader and encroach on the surface of the red dot until it was covered and converted into a white spot. The punctate hemorrhages, they thought, were more deeply situated and therefore less sharply defined, varied more in shape and size, often had a mossy outline and showed a tendency to absorption.

Because of my interest in the differential diagnosis of retinal micro-aneurysms, in their life history and in the minimal size required for ophthalmoscopic recognition, I asked some of my colleagues in the Section of Ophthalmology to answer 5 questions. In response to the query whether they thought that all of the punctate red spots in the retinas of diabetic patients were micro-aneurysms, Rucker[45], Henderson[28], Martens[39] and Hollenhorst[29] answered in the negative; Bair[7] answered affirmatively. With reference to distinguishing ophthalmoscopic features, all of my colleagues stressed the sharp demarcation of micro-aneurysms as contrasted to the poorer definition of the hemorrhages, though they admitted the difficulty of differentiating many of the individual lesions. Martens[39] and Henderson[28] thought it unlikely that a micro-aneurysm would disappear. Bair[7], Rucker[45] and Hollenhorst[29] thought that some of the micro-aneurysms might disappear; Bair[7] suggested as possible mechanisms of this disappearance a closing down of the efferent vessel or rupture of the micro-aneurysm. None of these observers thought that the waxy exudates of diabetic retinopathy represented hyalinized and obliterated capillary micro-

aneurysms. All of them agreed that, in most instances, a spot in the retina would have to be about 20 microns in size to be seen ophthalmoscopically, although Bair[7], Rucker[45] and Henderson[28] said that, under conditions permitting better contrast with the background, spots as small as 10 microns might be visible.

I think that the opinions just given represent a good cross section of the present views of practicing ophthalmologists on the question of retinal micro-aneurysms in diabetes. Personally, bearing in mind the results of measurements of the retinal vessels by means of the grid or the heliometer as reported by Koch[34], I am inclined to think that micro-aneurysms or red dots of about 10 microns in size should be readily visible in the presence of clear media. It is probable that the more deeply situated and more poorly defined punctate hemorrhages would have to be somewhat larger to be recognized.

Significance of the Concept of Retinal Micro-aneurysms. The importance of prevention or control, insofar as is possible, of the vascular lesions that endanger the vision and ultimately the life of the diabetic patient has appeared to warrant a review of the progress that has been made during the last decade in studies of retinal lesions in diabetes. This review is in some respects discouraging for, in the words of Ashton[2], "It is believed that it has not previously been realized how surprisingly numerous micro-aneurysms are and the picture is a depressing one, for one wonders how it can ever be possible to reverse such a gross and widespread process by the administration of drugs and the control of diet. At best we can only hope to prevent the development of such lesions or, once the condition is established, to attempt to control the hemorrhages."

Ballantyne[9] stated, "So far as the ophthalmoscopic diagnosis of diabetes is concerned, micro-aneurysms and micro-hemorrhages have the same significance; but as a contribution to the pathogenesis both of the local and of the systemic disease the differentiation of the lesions may be of the utmost importance." In this regard, a review of the accomplishments is encouraging. Friedenwald[26] has said, "The present state of the knowledge of diabetic retinopathy is still inadequate, but the work of many investigators during the past decade has served to define and describe the vascular lesion that characterizes this disease. It would appear that the happy and exciting threshold has been reached at which specific and potentially answerable questions can be formulated regarding the pathogenesis and etiology of this vascular disease."

REFERENCES

(1) **Ashton, N.**: Brit. J. Ophth., **33**, 407, 1949. (2) Idem: Ibid., **34**, 38, 1950. (3) Idem: Tr. 16th Internat. Congress Ophth., London, **1**, 411, 1950. (4) Idem: Brit. J. Ophth., **35**, 189, 1951. (5) Idem: Proc. Roy Soc. Med., **44**, 747, 1951. (6) **Ashton, N.**, and **Langley, D.**: Brit. J. Ophth., **35**, 424, 1951.

(7) **Bair, H. L.**: Personal communication to the author. (8) **Ballantyne, A. J.**: Arch. Opth., 33, 97, 1945. (9) Idem: Tr. Ophth. Soc. U. Kingdom, **66**, 503, 1946. (10) **Ballantyne, A. J.**, and **Loewenstein, A.**: Tr. Ophth. Soc. U. Kingdom, **63**, 95, 1943. (11) Iidem: Brit. J. Ophth., **28**, 593, 1944. (12) **Ballantyne, A. J.**, and **Michaelson, I. C.**: Tr. Ophth. Soc. U. Kingdom, **67**, 59, 947. (13) Iidem: Modern Trends in Ophthalmology (Sorsby), Paul B. Hoeber, Inc., New York, **2**, 262, 1947. (14) **Becker, B.**, and **Post, L. T., Jr.**: Am. J. Ophth., **34**, 677, 1951.

(15) **Day, R.**: Ibid., **33**, 224, 1950. (16) Idem: South Med. J., **44**, 549, 1951.

(17) **Edington, G.,** and **Sarkies, J. W. R.:** Tr. Roy Soc. Trop. Med. & Hyg., **46,** 59, 1952. **(18) Elwyn, H.:** Arch. Ophth., **33,** 315, 1945. **(19)** Idem: Ibid., **34,** 187, 1945. **(20) Evans, J. N.:** Ibid., **37,** 182, 1947.

(21) **Fraser, R., Simpson, C. L.,** and **Ashton, N.:** Overseas Postgrad. Med. J., **5,** 35, 1950. **(22) Friedenwald, J. S.:** Arch. Ophth., **37,** 403, 1947. **(23)** Idem: Tr. Am. Acad. Ophth., **53,** 73, 1948. **(24)** Idem: Am. J. Ophth., **32,** 487, 1949. **(25)** Idem: Ibid., **33,** 1187, 1950. **(26) Idem:** J. Am. Med. Assn., **150,** 969, 1952.

(27) **Heath, P.:** Discussion. Arch. Ophth., n.s. **24,** 754, 1940. **(28) Henderson, J. W.:** Personal communication to the author. **(29) Hollenhorst, R. W.:** Personal communication to the author.

(30) **Igersheimer, J.:** Bull. New England Med. Center, **13,** 264, 1951.

(31) **Jacobs, H. R.:** J. Lab. & Clin. Med., **34,** 116, 1949. **(32) Janes, R. G., Bounds, G. W., Jr.,** and **Leinfelder, P. J.:** Arch. Ophth., **48,** 414, 1952.

(33) **Keiding, N. R., Root, H. F.,** and **Marble, A.:** J. Am. Med. Assn., **150,** 964, 1952. **(34) Koch, F. L. P.:** Arch. Ophth., **34,** 321, 1945.

(35) **Lawrence, R. D.:** Proc. Roy. Soc. Med., **44,** 742, 1951. **(36) Loewenstein, A.:** Med. Klin., **46,** 936, 1951. **(37) Loewenstein, A.,** and **Garrow, A.:** Am. J. Ophth., **28,** 840, 1945.

(38) **Mackenzie, S.:** Roy. London Ophth. Hosp. Rep., **9,** 134, 1877. **(39) Martens, T. G.:** Personal communication to the author. **(40) Martin, M. M.:** Proc. Roy. Soc. Med., 44, 754, 1951. **(41) McCulloch, C.,** and **Pashby, T. J.:** Brit. J. Ophth., **34,** 495, 1950. **(42) Michaelson, I. C.,** and **Campbell, A. C. P.:** Tr. Ophth. Soc. U. Kingdom, **60,** 71, 1940. **(43) Michaelson, I. C.,** and **Steedman, H. F.:** Brit. J. Ophth., **33,** 376, 1949.

(44) **Nettleship, E.:** Tr. Ophth. Soc. U. Kingdom, **8,** 159, 161, 1888.

(45) **Rucker, C. W.:** Personal communication to the author.

(46) **Schneider, R. W., Lewis, L., Moses, J.,** and **McCullagh, E. P.:** J. Lab. & Clin. Med., **30,** 364, 1945. **(47) Scott, G. I.:** Proc. Roy. Soc. Med., **44,** 743, 1951. **(48) Sherrill, J. W.:** Bull. Scripps Metab. Clin., **2,** 1, 1951. **(49) Smith, H. E.:** Am. J. Ophth., **33,** 453, 1950. **(50) Sysi, R.:** Brit. J. Ophth., **35,** 560, 1951.

(51) **Wagener, H. P.:** Proc. Am. Diabetes Assn., **5,** 203, 1945. **(52) Weinstein, P.:** Ophthalmologica, **121,** 353, 1951. **(53) Wexler, D.,** and **Branower, G.:** Arch. Ophth., **44,** 539, 1950.

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PROCEEDINGS OF

The Physiological Society of Philadelphia

SESSION OF NOVEMBER 18, 1952

The early Detection of Experimental Pulmonary Edema in a Dog breathing spontaneously. DOMINGO M. AVIADO, JR., M.D., DENE WALTERS, and CARL F. SCHMIDT, M.D. (Dept. of Pharmacology School of Med., Univ. of Penna.). THE causation of pulmonary edema by increased hydrostatic pressure in the lung capillaries is emphasized by two known situations, namely, left ventricular failure (Welch, 1878) and pulmonary congestion by intense constriction of the peripheral vessels (Sarnoff, 1951). A third way of producing congestion by a local action on the lung vessels is suggested by the pulmonary edema that is induced by toxic chemical agents. The evidences for such a mechanism are as follows:

(a) The intravenous injection of alloxan (0.1 gm./kg.) or alphanaphthylthiourea (0.05 gm./kg.) into anesthetized dogs is followed by fatal pulmonary edema within 2 hours.

(b) There are no significant changes in carotid blood pressure and left auricular pressure to account for the appearance of tracheal froth.

(c) The important effects of the chemicals on the pulmonary vessels consist of pulmonary arterial hypertension and increased amount of erythrocytes (tagged with phosphorus$_{32}$) in the lung capillaries. This congestion and hypertension by pulmonary venular constriction is seen in the isolated perfused lung and in the whole animal.

(d) The earliest sign of edema consists of the accumulation in the lungs of plasma albumin tagged with iodine$_{131}$. This is detected by a Geiger tube on the pleural surface of the lung with a rubber membrane sealing the chest window.

These observations emphasize the fact that pulmonary arterial hypertension and capillary congestion can occur by a local action of chemicals on the lung vessels. The additional role of capillary permeability is under investigation.

Enzymatic Formation of Cyanide from Thiocyanate. FRANZ GOLDSTEIN, FREDRIC RIEDERS, Ph.D., and HEINRICH BRIEGER, M.D. (Dept. of Pharmacology and Division of Industrial Medicine, Jefferson Medical College, Philadelphia, Pa.) PREVIOUSLY published evidence (Goldstein and Rieders, Am. J. Physiol., **167**, 47, 1951) that cyanide appears in the blood of dog and man after administration of thiocyanate suggested the re-investigation of the reversibility of the "rhodanese" reaction. Rhodanese was prepared according to Cosby and Sumner (Arch. Biochem., **7**, 457, 1945). When it was incubated with KSCN alone or with the addition of methemoglobin as a CN^- acceptor, of sulphite as one end-product of the rhodanese reaction or of alanine as a possible sulphur acceptor, no CN^- formation was detectable by the method of Gettler and Goldbaum (Anal. Chem., **19**, 270, 1047). Organs of rats or rabbits poisoned with KSCN contained no CN^- or any traces of CN^-, the blood, however, contained *toxic* concentrations. Incubation of KSCN with whole blood, erythrocytes or plasma *in vitro* showed that CN^- is formed in the erythrocytes. Enzymatically active cell-free preparations were

obtained from red blood cells by precipitation with acetone and chloroform, dialysis against dilute phosphate buffer and precipitation with $(NH_4)_2So_4$. Temperature of 100° or iodoacetic acid caused irreversible inactivation. whereas inactivation by inorganic salts of heavy metals or by p-chloromercuribenzoic acid could be reversed with glutathione or cysteine. Hence, it appears that the conversion is enzymatic and that essential SH groups are involved. Preliminary studies of the kinetics of the reaction with hemolysates of erythrocytes indicate that the optimal conditions for the enzymatic conversion of thiocyanate to cyanide are 40°, pH 7.4, an incubation time of 1 hr. (85% of maximal amount of CN^- formed) and a substrate (KSCN) concentration of 0.075 M/liter. The amount of CN^- formed is also a function of the enzyme concentration. Under optimal conditions, 1 cc. of fresh erythrocytes forms about 10 gamma CN^-.

Internal Transfers of Water and Electrolytes in Acute Adrenal Cortical Insufficiency in Man. A. G. HILLS, M.D., and T. M. CHALMERS, M.D. (Dept. Medicine, University of Pennsylvania, Philadelphia). THE volume of inulin distribution was determined before and 2 days after withdrawal of adrenal replacement therapy in 6 patients who had previously had a subtotal adrenalectomy for severe hypertensive disease.

Five of the 6 patients manifested some chemical evidence of acute adrenal insufficiency, and all of the 5 exhibited a decrease in the volume of inulin distribution. The mean change for the 5 was —26 ±4.6%. There was a significant decrease in the total calculated sodium, chloride, and potassium in the inulin space, of which not more than 37% could ever be accounted for in the external balances.

There was a much smaller though still significant decrease in the chloride space amounting to 0.68 ±.16 liters. The water disappearing from the chloride space and the inulin space was not accounted for by the calculated external water loss. A net disappearance from the chloride space (after correction for the external balances) of potassium, but not of sodium, was observed. Together with other evidence, the data suggest that acute adrenal insufficiency in man is attended by transfer into a connective tissue compartment of fluid of a composition approximating that of extracellular fluid, and by a shift of water and potassium into cells.

Histochemical Localization of Cholinesterases in the Central Nervous System of the Rat. GEORGE B. KOELLE, PH.D., M.D. (Dept. of Physiology and Pharmacology, Graduate School of Medicine, Univ. of Penna.). BY a modification of a previously published method, the distributions of specific and non-specific cholinesterases (ChE's) were studied in the brain and spinal cord of the rat. The highest concentrations of specific ChE were found in the neurons of most motor nuclei and certain correlation centers; high concentrations were also observed in several tertiary afferent neurons. Moderate concentrations were noted in the second neurons of several sensory pathways and in other correlation centers; little or no activity was present in primary sensory neurons. certain neurons synapsing directly with motor neurons, and in other correlation centers. Nonspecific ChE activity was localized chiefly in the walls of the capillaries. the muscle fibers of arterioles and venules, and gliocytes (especially fibrous astrocytes); low concentrations of non-specific ChE also appeared to be present in certain neurons. Further details of localization and the relationship of the present findings to those obtained by other methods will be discussed.

BOOK REVIEWS AND NOTICES

FÜR UND WIDER DIE STERILISIERUNG AUS EUGENISCHER INDIKATION. By HANS NACHTSHEIM, Director of the Institute für Genetik of the Free University, Berlin, Prof. of General Biology and Genetics. Pp. 64; 4 ills. Stuttgart: Georg Thieme Verlag, 1952. Price, DM 5.70.

THE author blames the former Nazi regime that practically nothing has been done in Germany in the way of legislation to favor sterilization (vasectomy, salpingectomy) of individuals with seriously transmissible dysgenic deformities and diseases. In the East zone the Communist authorities advocate the doctrine that environment has everything to do with these dysgenic transmissible factors, heredity nothing. Religious influences may also play a part in moulding the opinions of well-intentioned medical authorities.

The author points to the advances in genetics made in the Scandinavian countries, especially Denmark, and to the statistics of results obtained in the United States, where at least 30 states have laws that permit sterilization. In certain countries even justifiable abortion is permitted.

In human beings dominant effects may be readily eliminated from families as the result of sterilization, although recessive defects are harder to outroot and may never be entirely got rid of.

Certain "phenocopies" simulate transmissible deformities, but are never such. They are produced by external agents and can be brought about experimentally in animals. In humans, the deformities produced in the fetus by rubeola and other viruses (and by certain infections in the mother) are cases in point.

There is no regeneration of affected genes in course of time; and sterilization is more effective and less expensive than custodial care in the elimination of defective genes.

Sterilization for the elimination of faulty genes should always be voluntary on the part of the affected person.

The effect of mutagenic factors (radiations, drugs, carcinogenic factors) are mentioned in a concluding section.

On the whole, the reviewer agrees with the author in practically all his statements.

Perhaps the author has included too many deviations in his setting forth of the hereditary transmissible defects in man. He points out that in spite of improved treatment of diabetes mellitus and of the lip-jaw-palate split, many of the offspring of diabetics die early, and that the incidence of the lip-jaw-palate defect has doubled during the past century. Hereditary epilepsy should be carefully distinguished from the symptomatic form. Schizophrenia is considered recessive from the genetic standpoint, cyclothymia dominant.

The author has presented his case well.

M. M.

ROENTGENOLOGY IN OBSTETRICS AND GYNECOLOGY. By WILLIAM SNOW, M.D., Chief Radiologist, V. A. Hospital, Shreveport, La. 2nd ed. Pp. 364; 305 ills. Springfield, Ill.: Charles C Thomas, 1952. Price, $10.50.

THIS second edition formerly titled "Clinical Roentgenology of Pregnancy" covers all aspects of Roentgen diagnosis in gynecologic and obstetric patients. A detailed discussion of methods and techniques for Roentgen pelvimetry and fetometry is presented in an interesting and easily comprehended manner. The studies of bony pelvic architecture and of the mechanism of labor as demonstrated by Roentgen-ray are of practical value and although the author's classification of pelves differs somewhat from the usual ones it is simple and useable.

The sections on placental localization and of soft tissue study are interesting and valuable since effort ordinarily is concentrated on visualizing only the bony pelvis.

Roentgen examination of the gynecologic patient includes bowel and urinary tract studies and is well presented.

The book is illustrated by reproductions of roentgenograms and line drawings which, for the most part, are well reproduced and demonstrate the structures adequately. The illustrations are separated from the text by many pages but due to the profusion of the reproductions this is unavoidable. When the author ventures into the field of clinical obstetric and gynecology he is apt to present ideas which have little to substantiate them; for example he attributes multiple symptoms to displaced uteri and states dogmatically the excess estrogen causes fibromyomata.

In general, however, this book succeeds in the author's aim that it "shall not only acquaint the reader with the Roentgen study of pregnancy but above all shall enable him to do his work."

J. W.

TRANSACTIONS OF THE CONFERENCE ON MORALE—AND THE PREVENTION AND CONTROL OF PANIC. Held under the joint auspices of the New York Academy of Medicine and Josiah Macy, Jr., Foundation. Pp. 75. New York: 1952. Gratis on request to Dr. Iago Gladston, New York Academy of Medicine, 2 E. 103rd St., New York 29, N. Y.

THE exigencies of the times demand deeper studies of the phenomena of Panic and Morale, said Dr. Galdston, at the Conference. In pointing up the counter-position of Panic and Morale, Dr. Galdston defined the former as primitive and disorganized and the latter as stemming from the word "moral" having to do with the right and wrong of human conduct.

Dr. Joost A. M. Meerloo former Chief, Psychological Department, Netherlands Army in discussing "People's Reaction to Danger," said that if a population is well prepared and morale is good, jitters will appear in everyone but last only a short time. During World War II, he said, fear reaction paradoxically often appeared and had to be dealt with *after* the danger had passed. Two types of panicky behavior were most common (1) an apathetic paralysis and (2) childlike rage reactions resembling temper tantrums that included scolding and crying for help and even aggressive behavior. Two psychosomatic reactions were common (1) a vasoconstriction causing pain in various parts of the body and (2) urinary frequency. Scolding those in panic, he added, does not help, nor do narcotics. Coffee, soup, food, cigarettes are more effective. For more prolonged anxiety reactions, hypnocatharsis and narcoanalysis were used.

Herbert Brucher, Editor, discussed the spread of rumor that arises without sources of reliable information such as newspapers and radio. He warned against the use of either to manipulate public opinion but felt they should stick to the truth. Sound policy, he said, will create its own good public relations. Robert Saudek, Vice President in charge of public affairs, American Broadcasting Company, who represented radio communication in relation to Morale and Panic, also stressed the necessity of checking on the accuracy of news broadcast.

Dr. George S. Stevenson, Medical Director, The National Association for Mental Hygiene Inc., discussed the role of the voluntary and official agencies in the promotion of Morale and the prevention of Panic. He pointed out the fact that at the time of an emergency, people will turn to an agency they know. An effort should be made to make people aware of the various agencies existing and their specific functions. There should be a basic competence in all agencies, he added, to deal with human problems. Their common generic function should involve (1) the capacity to interview (2) an understanding of anxiety (3) an understanding of family relations (4) a knowledge of community services and how to use them.

Dr. James Stewart Tyhurst, member of the Defense Research Board, Ottawa, Canada, stressed how little is known of the factors which affect Morale and Panic and stressed the need for more research. Dr. Leo Alexander, Tufts Medical School, in discussing Morale and Leadership, said, "In general terms, morale may be defined as the ability to maintain a high degree of optimism and purposeful activity in the face of adversity. A leader must see to it that individuals in the group take responsibility for executing the group's aims. In order for subordination of individual interest to occur, some sort of compensatory gratifications must be offered. The two most important compensations are (a) personal pride and investment in the accomplishment of a common purpose, and (b) gratification through identification with the powerful leader. The leader must place the good of the group before his personal satisfactions, often subordinating his personal interests even more than do other members of the group. He must have no personal axe to grind."

Every physician should be able to put to good use information on Panic and Morale and this report is highly recommended.

O. E.

ÜBER DEN WAHN. BY KURT SCHNEIDER, Professor at the University of Heidelberg. Pp. 48. Stuttgart: Georg Thieme Verlag, 1952. Price, DM 3.90.

THIS is an analysis of the subject of "delusion," especially from the standpoint of the German literature and with respect to the author's individual views. He defines "delusional perception" as an actual perception that acquires abnormal significance without rationally or emotionally intelligible occasion. These perceptions must be distinguished from those with intelligible false significance that arise on the basis of anxiety, mistrust, suspicion and the like, which are not really delusions but delusion-like experiential events or reactions. Delusions often accompany schizophrenia, but schizophrenia may also be accompanied by delusion-like reactions. We also may speak of delusional vagaries (Ein-

faelle) in which there are overestimations as to religious or political vocations, or especial ability, or persecution, or as to being loved. True delusion should be differentiated from the experience with regard to symbols of the non-psychotic life (such as the finding of a four-leaved clover, and the like). In delusional vagaries the delusional element is one-membered (that is, lacks the abnormal significance), and has no specific structure.

The actual practical differentiation of these various forms of delusional elements is very difficult indeed. As additional elements are mnestic delusions and delusional vagaries in which actual perceptions in the more or less distant past acquire a special significance, or one-membered special abilities and the like are referred to this past. Delusions and delusion-like reactions of psychotics which are used by them as the basis of persistent opinions, as well as persistent delusional vagaries, are known to us as delusional thoughts. When there are connections fabricated between the various delusional elements just mentioned, we have what we know as a delusional system.

In the second (clinical) portion, the author speaks of the difficulty in diagnosing the type of psychosis (or neurosis) from the delusion alone and the fact that it is difficult to determine whether certain delusional trends are due to personality or represent real psychotic stigmata. He believes that the characterogenic view of the accounting for delusional states really explains or makes intelligible only the theme or contents of the delusion but not that a delusion is actually present. In the determination whether a delusion is truly such or is due in the case at hand to personality factors, the theme is to be distinguished from the "Sinneweise" (form).

The delusional-like spells in alcoholism, anxiety, brain injury, uremia, diabetic coma, and the so-called "induced psychosis," twilight states of epileptics, are mentioned and commented upon. Chronic delusional alcoholic psychosis is now usually discarded as a diagnosis by psychiatrists. Two forms of psychosis take its place in the nomenclature: schizophrenia and alcoholic Korsakow's psychosis, both of which are regarded as exogenous. In addition, the author considers paranoid psychosis in the course of anemia; also delusional states in the course of dementia, progressive paralysis (psychotic), senility, and cyclothymia. The idea is expressed that schizophrenia and cyclothymia are merely types of the endogenous psychosis.

Schizophrenia is the psychosis which is attended with delusions in the narrow sense. When there are delusional vagaries only we may speak of a paraphrenia (or of a paranoia) in which case we understand a schizophrenic psychosis in the broader sense. He agrees with Kraepelin's point of view that paranoia bears more the stamp of a diseased development than of a process of mental illness distorting and destroying the personality. The existence of a paraphrenia is never derivable from the personality and its experiential events.

The reviewer regards the pamphlet as a very worthy piece of keen analysis (not psychoanalysis), which should be read to be appreciated fully. M. M.

TEXTBOOK OF GYNECOLOGY. By EMIL NOVAK, A.B., M.D., D.Sc. (Hon.), F. A. C. S., F.R.C.O.G. (Hon.) and EDMUND R. NOVAK, A.B., M.D., F.A.C.S. 4th ed. Pp. 800; 523 ills. Baltimore: Williams & Wilkins Co., 1952. Price, $9.00.

THE purpose of the earlier editions to present gynecology in a simple practical manner has been retained in this 4th edition of Novak's Textbook of Gynecology now edited by the original author and his son. Many portions have been rewritten, the number of pages has been increased but the chapter headings remain the same.

The first 11 chapters concern themselves with the anatomy, physiology and anomalies of the genital tract while the remainder discuss specific disease conditions arranged in general according to the structure involved. The actions of the various hormones on the pelvic organs are described and the use of endocrine preparations in clinical gynecology is presented throughout the text and summarized in the section entitled General Principles of Gynecologic Organotherapy. Unfortunately they give the impression that certain preparations such as chorionic and equine gonadotropins have a definite place in therapy despite the evidence against it. No operative procedures are presented. Novak's interest in gynecologic pathology is evident in the many illustrations of microscopic anatomy, many of which lose their value because they are poorly reproduced. Many could be eliminated without subtracting from the value of the text; in fact an increase in the number of illustrations of gross pathologic specimens and of methods of clinical examination and treatment would increase the practicality of the volume.

The authors' aim to provide a practical textbook of gynecology for the medical student and practitioner is achieved in general but often no definite decision is made

for the reader as to the best of several suggested methods of therapy.

An indication by the authors concerning their own experience rather than those recorded by others would improve the text. It remains however one of the best of the gynecologic texts for those for whom it was intended. J. W.

CULDOSCOPY. BY ALBERT DECKER, M.D., D.O.G., F.A.C.S., Clin. Prof. of Gynec. & Obst., New York Polyclinic Medical School. Pp. 148; 50 ills. Philadelphia: W. B. Saunders Co., 1952. Price, $3.50.

IN the preface to the monograph "Culdoscopy" the author states that the entire technique and all the special diagnostic and therapeutic procedures will be discussed in detail. This he is eminently qualified to do since the method, which had been used occasionally for many years, has recently been perfected by him.

This book includes a detailed history of the endoscopic examination of the peritoneal cavity with particular reference to culdoscopy and a clear concise description of the local anatomy.

The technique of cul-de-sac puncture and of culdoscopy are described in great detail and their use and limitations as diagnostic procedures in pelvic disease are presented. The therapeutic uses of the procedure particularly in reference to the production of pneumoperitoneum and in infertility also are described.

The text is well illustrated by drawings and a few roentgenograms and photographs which are well reproduced and illustrate the subject material adequately. In general the monograph accomplishes its avowed purpose of acting "as a guide to the gynecologist who desires to employ culdoscopy." J. W.

KYMOGRAPHISCHE RÖNTGENDIAGNOSTIK ZUR BEURTEILUNG DES HERZENS IN BEISPIELEN. By DR. P. STUMPF, Munich. Pp. 120; 164 ills. Stuttgart: GEORG THIEME, 1951. Price, DM 25.50.

DR. STUMPF is one of the pioneers in the field of Roentgen-kymography, with particular reference to the heart and great vessels. In the introduction, the technique is outlined and it is properly pointed out that in evaluating the registration of the movements of the silhouette contour one has to take into consideration the phases of filling and the pendulum movements, also, though they are less significant, the rotatory movement of the heart and the cranio-caudal motion of the apical portion. Alterations in the density of the heart shadow may be usefully correlated with the observations on marginal movements.

In forty different cases short clinical, and occasional electrocardiographic (limb leads) data are given, followed by the kymographic illustration, diagnosis, and a detailed evaluating discussion. The type of conditions represented are: normal; valvular lesions; myocardial infarctions; enlargement of the heart, the etiology of which is not always obvious; myocardial insufficiency; auricular fibrillation and ventricular premature beats; aneurysms of the aorta; mediastinal tumors; pericardial disease (effusion, calcification); metallic foreign bodies. Postmortem data are available in a few cases only.

It is demonstrated in some instances that the kymograms may be normal under resting conditions, but may reveal abnormal findings following exercise.

Some of the author's conclusions are somewhat intuitive in character, and may not readily be duplicated by others, particularly by those who have not had a great deal of experience with the method.

The illustrations and printing are excellent, as one would expect from the old reputable German publishing house of Thieme.

H. R.

PRINCIPLES AND METHODS OF PHYSICAL DIAGNOSIS. BY SIMON S. LEOPOLD, M.D., Associate Professor of Clinical Medicine, University of Pennsylvania School of Medicine and Graduate School of Medicine. Pp. 430; 390 ills., and 19 color plates. Philadelphia: W. B. Saunders Co., 1952. Price, $7.50.

THIS is an excellent text on physical diagnosis and well suited to the Sophomore Medical Student or anyone who wishes to review fundamentals of physical diagnosis. The material is well organized and is accompanied by excellent illustrations and charts. A chapter of Acoustic Principles by S. Reid Warren, Jr., Sc.D. in E.E., gives the fundamentals very simply and these are correlated with the practical methods of physical diagnosis. There are three other points which, to the reviewer's mind, make this a modern and progressive text. One is a chapter on examination of the breast which is quite thorough and again accompanied by excellent illustrations. Another is on the examination of the musculoskeletal system which in the manner of its presentation is new to texts of this sort and is an excellent guide to a thorough and complete examination of this system. A chapter dealing with the psychiatric survey is in

line with the progress of medicine and a good time to present this important phase of diagnosis to the student.

The rest of the book covers the usual material which is included in most of the texts of this nature, but its organization is such that it is simple, yet complete.

G. M.

LIVING AGENTS OF DISEASE. By JAMES T. CULBERTSON AND M. CORDELIA COWAN, Columbia University. Pp. 606; 71 ills. and 24 tables. New York: G. P. Putnam's Sons, 1952. Price, $5.50.

THIS textbook is really a primer of microbiology and public health in its broadest aspects. Although the style is breezy and the treatment superficial, the content appears to have been selected with great care. A unique feature is the inclusion of collateral topics such as the role of government in medicine and public health, federal and local regulations, as well as material which can best be described as medical civics and economics. Those charged with the instruction of students in nursing, medical technology and any category of public health should examine it for the fresh approach it uses.

E. S.

ARMY MEDICAL LIBRARY CATALOG, 1951. PART 1: AUTHORS; PART 2: SUBJECTS. Pp. 866. Washington, D. C.: Library of Congress, 1952. Price, $17.50.

THIS important bibliographical tool, issued annually, lists the monographic titles added to or re-catalogued by the Army Medical Library (now the Armed Forces Medical Library) in the preceding year. The catalogue and its companion publication, the *Current List of Medical Literature*, an index to the most important journals received by the library, superseded a few years ago the library's famous *Index-catalogue*.

The new volume has at least one new feature that will be widely acclaimed. In place of what had been merely a subject index to the author section, there is now a subject section which repeats the bibliographic information formerly found only in the author section. Some users may find the resultant saving in time partially offset by the reduced type-sizes needed for economic reasons and to keep the volume as easy to handle as in the past; but the majority will probably find the net gain a considerable one. Fortunately, this improvement has not resulted in an increase in the purchase price. While the price does not appear to be unreasonable, all factors considered, it is regrettable that it is still probably high enough to limit the distribution of a working tool that ought to be available to everyone needing recourse to the medical literature.

Research workers in the medical sciences, and the practitioner with an old or new area to survey, have here renewed occasion to be grateful for the continuing bibliographical assistance provided by our national medical library.

W. McD.

BASIC MEDICAL PHYSIOLOGY. By W. B. YOUMANS, Ph.D., M.D., Prof. of Physiology, University of Wisconsin, Madison. Pp. 436; 117 ills. Chicago: The Year Book Publishers, Inc., 1952. Price, $7.50.

THE author is well qualified both by training and experience to carry out the intent of this book. This work is a concise presentation of physiological principles. These principles can be used as a foundation upon which to build the information needed for a study of physiology and pathology which is abnormal physiology. This book will make an excellent text for those contemplating the subsequent study of Medicine or Medical Sciences. It would also be useful as a review text for medical students. The figures are largely new and the bibliography concise and extremely well chosen.

M. O.

STUDIES IN VISUAL OPTICS. By JOSEPH I. PASCAL, B.S., M.A. O.D., M.D. Attending Ophthalmologist, New York Polyclinic Medical School and Hospital. Pp. 800, 138 ills. St. Louis: C. V. Mosby Co., 1952. Price, $12.50.

PRACTICING ophthalmologists cannot well afford to fail to have mastered Dr. Pascal's new book, which represents a review and compilation of his 150-odd journal contributions to the science of refraction. The book enters the serious gap in ophthalmic science, that lying between medical or surgical practice and physiological optics. The author has organized past knowledge and his own discoveries into a methodology of refraction replete with practical examples and complete in bibliographical references. Although the book may appear rather long to somewhat lazy readers, one must recall that Dr. Pascal has an earned reputation as both literator and poet. The book is pleasant to read. It is a practical treatise on geometric optics, dynamic retinoscopy, lens effectivity, refractive correction, accommodation-convergence relationships, orthoptic training, and heterophoria. It is recommended as a text for students, and as a practical guide for ophthalmologists.

R. P.

NEW BOOKS

The 1952 Year Book of General Surgery. Edited by EVARTS A. GRAHAM, M.D. With Section on Anesthesia edited by STUART C. CULLEN, M.D. Pp. 670; 203 ills. Chicago: Year Book Publishers, 1952. Price, $6.00.

THIS perennial review of the literature on surgery and anesthesia is up to its high standard of excellence. R. K.

Infrared Photography in Medicine. BY LEO C. MASSOPUST, SR., Director, Dept. of Art and Photography, Marquette University School of Medicine. Pp. 53; 31 ills. Springfield, Ill.: Charles C Thomas, 1952. Price, $2.75.

INTENDED as a stimulus to encourage further application of infrared photography to medical subjects, this little monograph summarizes briefly the differences in method from those of ordinary photography. Facing illustrations of identical subjects (gross specimens, photomicrographs and clinical patients) as recorded by conventional film, and by infrared sensitive emulsion, strikingly support the author's case for the latter; especially for studies of the vascular system. N. B.

Cortone. A Handbook of Therapy. Pp. 125; 31 ills. Rahway, N. J.: Merck & Co., Inc., 1952. Free on request to Box 22, Merck & Co., Inc., Rahway, N. J.

AN excellently arranged summary of the good effects of cortisone (with very little information on its bad effects) and with detailed advice as to indications and techniques of administration. J. L.

The Prevention of Rheumatic Fever. BY LOWELL A. RANTZ, M.D., Associate Professor of Medicine, Stanford University. Pp. 66. Springfield, Illinois: Charles C Thomas, 1952. Price, $2.25.

THE title of this monograph could readily be amplified to "The Etiology, Epidemiology and Prevention of Rheumatic Fever." Dr. Rantz, who has conducted many investigations in this field, authoritatively summarizes the evidence for the etiologic role of the streptococcus in rheumatic fever. The clinical recognition, management and prevention of streptococcic infections are delineated.

This monograph is recommended as pleasant and informative reading for the practicing clinician upon whom the burden of responsibility falls for preventing rheumatic heart disease, one of the leading causes of death and crippling illness in the young. W. S.

Glands, Sex and Personality. BY HERMAN H. RUBIN, M.D. Pp. 205; 2 ills. New York: Wilfred Funk, Inc., 1952. Price, $2.95.

THIS is another book written for the laity on the subject of endocrinology. W. P.

Biochemistry and Human Metabolism. BY BURNHAM S. WALKER, M.D., PH.D., Boston University School of Medicine; WILLIAM C. BOYD, PH.D., Boston University School of Medicine; and ISAAC ASIMOV, PH.D., Boston University School of Medicine; Foreward by JOHN T. EDSALL, M.D., Harvard University. Pp. 812; 21 ills. Baltimore: The Williams & Wilkins Company, 1952. Price, $9.00.

THIS new text is designed primarily for medical students. The authors have chosen a presentation varying from the usual, the volume being divided into six parts, with subtopics treated as chapters under each part: I. Structure, (1) proteins and amino acids; (2) protein structure, (3) tissue chemistry, (4) blood and the anemias; II. Control, (5) enzymes, (6) hormones; III. Growth, (7) nucleoproteins and growth, (8) cancer, (9) reproduction and heredity; IV. Metabolism, (10) food and diet, (11) digestion, (12) carbohydrates and diabetes, (13) lipid metabolism and ketosis, (14) proteins and starvation, (15) electrolytes and water: edema and shock, (16) respiration and acidosis, (17) heat and work, (18) excretion and some of its disturbances; V. Pathology, (19) vitamins and vitamin deficiency diseases, (20) infection; VI. Appendices, (I) colloids and colloid phenomena, (II) isotopes, (III) principles of thermodynamics, (IV) acids and bases.

Proteins, considered "of prime importance to medical biochemistry" are taken up first and treated extensively and well. Carbohydrates and lipids are treated in the introduction to tissue chemistry. One may question the placing of the vitamins under "Pathology." Inclusion of a 41-page chapter on "Reproduction and Heredity" is illustrative of the indefiniteness of the boundary between biochemistry and physiology. The reviewer considers the treatments of body buffer systems, of the chemistry of respiration, and of the chemistry of acid-base disturbances, inadequate for medical students. R. H.

NEW EDITIONS

Physical Foundations of Radiology. BY OTTO GLASSER, PH.D., EDITH H. QUIMBY, SC.D., LAURISTON S. TAYLOR, PH.D., AND J. L. WEATHERWAX, M.A. 2nd ed. Pp. 581. New York: Paul B. Hoeber, Inc., 1952. Price, $6.50.

EIGHT printings of the first edition since its publication eight years ago have attested to the popularity and demand for this concise orientation in the physical components of radiology. During this period, startling advances in the realms of radioactivity have occasioned revision and augmentation of the initial edition. The scope of the current volume has been broadened by the inclusion of new chapters on radioactive isotopes, supervoltage generators and nuclear reactors. The well-established Roentgen-ray dosage tables have been expanded and new data for linear radium sources have been included. As in the past, the book will continue to serve as a constant source of information to the radiologist and others concerned with the handling and use of radioactive material. R. S.